

It's your journal

"We would be happy to publish your letter if you could cut it in length by about 30%."

"We could publish your communication at letter length of about 1,000 words but not as a 3,000-word article."

"We regret it is almost impossible to publish an article of 6,000 words."

"We regret that your letter seemed too specialised for a journal aiming at a wide readership."

These are four of the types of letter that we don't like sending any more than you like receiving. For some years *Nature* has been publishing communications in the form of commissioned articles or reviews, research articles and letters. Looking back further, however, one sees that this format has only been reached by an evolutionary process; indeed the early issues of *Nature* contained an extraordinary diversity of material which the editor did little to segregate. Quarrels, opinions, anagrams and observations of atmospheric phenomena rubbed shoulders with announcements of scientific progress. Presumably those with a serious message did not have as great an objection as present-day scientists would to their scientific wheat sitting amongst anecdotal chaff—probably because they knew that next time they wrote it was as likely as not to be an anecdote that they sent.

In the past year we have tried to continue to evolve. A section has been started in which scientists can raise, briefly, points relating to earlier papers in *Nature*. (Though not too much earlier—about a year after publication we have to assume that ideas floated in our columns are starting to go through the normal refining process in the specialised literature and need no corrective action from us.) Further we occasionally take a more general communication and publish it in News and Views, particularly when it raises a controversial point of broad interest. Finally, we try to include after the leader a piece concerned with broader issues of science—policy, politics, method and so on. As often as not this will have been submitted originally as a letter. We are always pleased to see potential material both for News and Views and for the 'broader issues' pages, although the criteria by which we accept or decline have to be fairly subjective.

Although we have some instinct for what should go into these sections of the journal, and we expect that referees will have an instinct for the accuracy and general significance of the technical content of the papers communicated to us, we have a very limited ability to tell whether our present division into articles and letters and our present prescribed maximum lengths are in tune with authors' and readers' needs. Of course, we have (quite conflicting) opinions in the *Nature* office, and, of course, we do discuss the matter on occasions with scientists. But we lack any sort of market-place response. The number of subscribers who will cancel their subscription simply

because they find articles too specialised or letters too telegraphic is probably few. Again, it is difficult to judge whether a particular paper goes to another journal because it could not be condensed to a thousand words, because of the delay time in publication (though watch ours drop) or for any of half a dozen other reasons.

Because of all this, we take the perhaps foolish step of inviting opinions on how the medium could change the better to proclaim the message. There are some constraints. The upper limit of 3,000 words and six displayed items could not be revised upwards, simply because very long articles restrict the spread of interests in any particular issue and cut the acceptance rate for other manuscripts. Further, any policy by which the average length of letters is increased necessarily means that fewer can be published, as the number of pages that we can produce is unlikely to grow under present economic conditions. We do not, however, believe that the present acceptance rate of about 35% is an unalterable number.

Granted this, should we publish less or more papers of article length? And should we require that an article be particularly accessible to the general readership or would this destroy an important means of scientific communication? Should the distinction between articles and letters (a frequent bone of contention amongst authors cunningly submitting an 1,800-word paper) be abolished? Should the length limit for letters be raised—or even lowered?

We do make two pleas. First, that many authors have an ability to write to the limit whatever it may be and do not see it as a challenge to say in 600 words what might be expanded to 1,000. We shall continue to try to keep papers short and to cut out material which would be appropriate to a specialised journal but which gets in the way of the reader with limited time attempting to find out if there is anything in it for him. It is undoubtedly true that the shorter the paper the better the chance it has of being read widely.

Second, many papers have a depressing opacity which could easily have been alleviated before they were sent. If more authors would try out manuscripts on colleagues in different disciplines before submitting and would attempt to make the first paragraph into a crystal-clear description of what the paper is about rather than what other people's papers have been about, *Nature* would be an easier journal to read.



M. MARTIN, a French telegraphic engineer, has invented an engine for recording votes. The contrivance has been designed on the principle of the *sonnettes électriques*, and is exhibited in a shop in the Place Dauphine. The peculiarity is that the votes are registered and their total reckoned automatically. The invention is attracting public notice, as it is expected that the Versailles representatives will have an immense number of votes to register during the next session.

From *Nature*, 11, 94, December 3, 1874.



For those in peril: 3 - not only on the factory floor

Dr D. R. Bowes of the Department of Geology at the University of Glasgow points out that not only workers but the general public could be at risk from asbestos and other mineral dusts in the environment and that more research is needed on this particular topic.

THE discussion by Peter J. Smith of occupational exposure to asbestos (*Nature*, October 18) highlights a major obstacle to a concerted effort by the scientific community in the field of the relationship of mineral and rock dusts to human disease, namely the apparent lack of awareness of the problems by many mineralogists, geochemists and geologists and hence of the neglect of their expertise. Some are involved but, however significant their contributions may be, it has been a relatively small group of the medical profession who has pinpointed the vital role of mineralogy and allied fields as well as demonstrated the health hazards associated with the inhalation of mineral and rock dusts. Much of the recent direction of public attention to the matter stems from an articulate few who see not only the value of scientific research to community health, but also to social and legislative action. They have been supported, particularly in the United States in recent years, by active collaboration with physical scientists, which has not only contributed to medicine but has opened frontiers in the physical sciences. In Britain it is more than 40 years since an industrial survey showed the high prevalence of asbestosis in asbestos factory textile workers, and that came nearly a quarter of a century after Parliament was first notified, in 1906, about fatal pulmonary asbestosis amongst asbestos workers. With such a passage of time, and its implications for human disease and suffering, the focusing of attention of physical scientists on occupational exposure to asbestos, and to the many other mineral and rock dust materials associated with mining, milling and manufacturing industry, is a matter of urgency.

Urgent attention should also be given to potential health hazards concomitant with environmental exposure. The incidence of pleural calcification resulting from environmental exposure in the neighbourhood of an asbestos mine or mill, or of pleural mesothelioma, without occupational exposure, in areas where there is asbestos in the local rocks, serves to illustrate that potential



Not the only one at risk

risk is not limited to occupationally exposed miners and industrial workers. The 1971 Report of the National Institute of Occupational Diseases, Johannesburg records that more than a third (of 210) of cases of mesothelioma were considered to be the result of environmental and not occupational exposure to asbestos. This, together with evidence that asbestos greatly multiplies the already considerable lung cancer potential of cigarette smoking, suggests that combined scientific and medical research as a basis for legislative attention, as well as preventive medicine, could have a significant effect on community health in at least some industrial areas.

The dimensions of any health hazard associated with air pollution caused by dusts, including asbestos, have yet to be defined and it is not proper to equate neighbourhood contamination with general community air pollution. The existence of levels of 10 to $50 \times 10^{-9} \text{ g m}^{-3}$ of chrysotile asbestos in the ambient air of places like New York City and the regularity with which asbestos fibrils are found at autopsy in the lungs of residents of New York and London, are probably indicators for other urban areas. The asbestos industry has an important responsibility for controlling the commercial and industrial sources of air pollution, but additional possible contributory factors, resulting from the action of the public at large, must not be passed by. These include dusts from asbestos brake drum linings and the mineralogy of decomposed lining material which could have a general effect as well as a localised one in the confined spaces of motor repair premises; inorganic particles in smoke from tobacco—that from some cigars is known to contain an inorganic fibrogen and that from cigarettes, is suspect in view of the relationship between cigarette smoking and lung can-

cer; and consumer talc products, which must represent one of the mineral dusts most inhaled and ingested by people of all ages in many households, with talc known to cause pneumoconiosis as the result of occupational exposure and to occur in nature together with some asbestiform minerals.

There are other factors to be considered in the control of environmental exposure to mineral and rock dusts through air pollution, and many other geological and mineralogical aspects to be assessed in the relationship between human health and the inhalation and ingestion of inorganic particles. And those instanced now under joint or separate investigation in the Environmental Science Laboratory, Mount Sinai Hospital, New York, and the Department of Geology, University of Glasgow show not only how in science something that is apparently obvious, with the benefit of hindsight, is not recognised until its possible effect on human welfare is appreciated, but also how in developing fields the availability of equipment that can help meet the new demands is vital. Identification of gaps in existing knowledge as suggested by Peter J. Smith is a useful next step but consideration of environmental exposure to mineral and rock dusts, as well as occupational exposure, is likely to reveal larger gaps than initially anticipated and to suggest that inclusion of matters affecting the health of the public at large will entail interdisciplinary endeavour on a considerable scale. Review and assessment alone will, however, not avert human suffering but could lead to frustration on the part of scientists and cynicism on the part of the general public if the will to accomplish is not matched by adequate provision of the tools needed, even if this means that certain other research topics, with less application to human suffering, are given reduced support.

international news

Flowers to head new European Science Foundation

THE European Science Foundation took a large step forward in Strasbourg last week with the election of officers and the creation of an executive council. Sir Brian Flowers, physicist and Rector of Imperial College, was elected President and the two Vice-Presidents are Professor O. Reverdin, a Greek scholar and Chairman of the Swiss Fond National and Dr P. Riis, a Danish physician. Secretary-General of the ESF will be Dr F. Schneider, at present Secretary-General to the Max-Planck Institut in Munich. Dr Schneider will work part-time in Strasbourg from January 1975 and eventually will be employed full-time by the foundation. Other officers will attend in Strasbourg for a couple of days a month on average, particularly for the meetings of the council of eighteen members. Britain has a second member on the council, Dr S. G. Owen of the Medical Research Council.

The ESF emerged as a concept when it became clear that there was considerable resistance to the idea of a pure research organisation being based on the EEC. Two of the most commonly heard arguments were that an ESF based on the Nine would be tied by the ponderous political procedures of Brussels and that learning knew no frontiers. Accordingly, and with the EEC's blessing, responsibility for the encouragement of European collaboration in the furtherance of research was passed on to an ESF comprised of representatives of almost all Western European countries, and there was a clear requirement that the body should have flexibility.

The word 'Science' in the title is interpreted in its broader European context of learning or knowledge. The 43 member organisations which sit in the assembly, the governing body of the ESF, thus comprise not only funding agencies such as research councils of the natural and medical sciences but also academies both of science and the humanities. The British seats in the assembly are occupied by the five research councils, the Royal Society and the British Academy.

The budget for the first year is to be 2.2 million French francs, most of which will go in administrative expenditures in Strasbourg; it is not envisaged that the ESF will become a funding agency. Rather it is seen as an enabling organisation, providing a forum for in-

ternational discussions on collaborative projects. There is to be no requirement that all members should agree on these projects; on occasions the ESF may simply serve as a template on which two or three research organisations in different countries can put together a scheme for which they have funds.

The success of ESF will clearly depend on its ability to show its useful-

ness within the first year or two, and Sir Brian already has a list of projects to which the council will give early consideration. These are:—

Astronomy This is seen as a top priority as it is a natural field for multi-lateral collaboration and any consequent trimming of large national budgets on astronomy would be a big plus for the young ESF.

Archaeological techniques Clearly the archaeology of various parts of Europe differs widely, but techniques are much the same everywhere. The pooling of equipment and facilities could be the first step into the humanities for the ESF.

Comparative legal systems Getting to know how different countries work is going to be part of the European experience of the next ten or twenty years.

Social responsibility in biology Does this have a European dimension? ESF as yet does not know, but EMBO will be consulted early on the plasmid engineering issue.

Outside comment on large institutions Sir Brian would like to see ESF playing the same role in Europe as the National Academy of Sciences has done in the United States in providing informed comment and criticism on organisations such as ESRO. This is bound to be a fairly controversial proposal and he has already been presumed by some to be seeking to get at ESRO. Does ESF yet have the weight for this sort of operation?

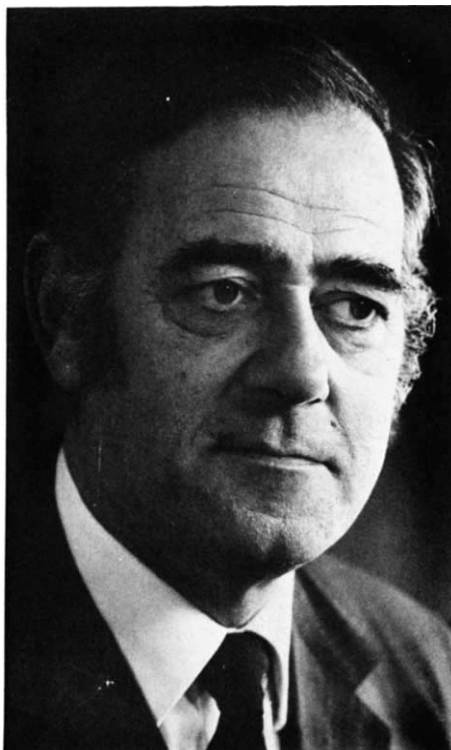
Marine biology Ireland is particularly keen to advance in this field.

Science policy There is thought to be scope within the ESF for discussions on the development of a more pan-European approach to policy-making.

Supporters of the ESF are at pains to point out that one of the first jobs of the foundation is to establish good working relations with the already-existing European-wide science and medical research council collaborative efforts. Since it is these councils at a national level which have been, *inter alios*, responsible for setting up the ESF this should not be a major difficulty.

In the longer run the ESF is seen as a body which national governments and international organisations will consult as a matter of course, if not as a legal necessity, on major matters of science and learning. □

Sir Brian Flowers



New Astronomer Royal for Scotland

by John Gribbin

THE announcement that Dr V. C. Reddish is to succeed Professor H. Brück as Astronomer Royal for Scotland and Director of the Royal Observatory, Edinburgh (ROE) comes as no surprise to astronomers. Dr Reddish's new post is one of the two key jobs in the rebuilding of British optical astronomy (see *Nature*, **251**, 456; 1974). The Royal Greenwich Observatory (RGO), under the Directorship of Professor F. G. Smith, will have responsibility for running the new Northern Hemisphere Observatory, whereas the ROE provides the base in the United Kingdom for the Science Research Council (SRC) team running the 48-inch Schmidt Camera at Siding Spring and also plays a large part in the Anglo-Australian Telescope project. In addition, the ROE has the responsibility of setting up the 3.8-m infrared flux collector which is to be built in Hawaii with SRC funds (see *Nature*, **250**, 617; 1974). So the responsibility for the hardware of what Professor Smith sees as a new 'Golden Age' of astronomy is divided evenly between Britain's two principal optical observatories.

Like Professor Smith at the RGO, Dr Reddish seems well qualified for the



task. His experience is rather more diverse than that of most academics, embracing 12 months in a brewery, three years in a bank and three years in the Royal Navy before he took his first degree. Since gaining his PhD as a member of University College, London, in 1954 Dr Reddish has been based in Edinburgh except for three years from 1959, which he spent at Jodrell Bank.

He says that those three years were

of great importance since they introduced him to the teamwork approach used by radio astronomers; with the electronic equipment attached to telescopes becoming ever more sophisticated there is now very little difference between the tasks of observers at radio, optical and other frequencies, and Dr Reddish realised from his experience at Jodrell Bank that modern astronomers would have to follow the same path of collaboration in order to get the best results out of their new instruments.

Since 1962 Dr Reddish has worked from time to time as a visitor at Hamburg, in the United States and in Australia; he was Project Officer for the construction of the United Kingdom Schmidt in Australia, and serves at present as one of three British members on the AAT Board. It is interesting that the future of British optical astronomy is largely in the hands of one man who has 'converted' from radio astronomy, and another who says that he learnt a great deal from his three years at Jodrell Bank. But divisions between observers today mean very little, and the real importance of both these appointments is that the men concerned have the proven administrative experience, as well as appropriate scientific pedigrees, to ensure that full advantage is taken of the opportunity now presented to British astronomy.

THE Soviet Mars mission of 1973-74 was only a partial success, with one intended satellite (Mars 4) and one landing craft (Mars 7) overshooting the planet. Results of the data from the more successful probes (*Zemlya i Vselennaya*, No. 5, 7-11; 1974), although scientifically stimulating, may well give certain embarrassment to Soviet politicians, for—in spite of their predilections for the colour—the red planet is, in fact, not quite as red as it has previously been painted.

According to the colour photographs received from the orbiting Mars 5 (and from the overshooting Mars 4), the craters in particular reveal significant variations of colour. Some of them exhibit a blue-green tint, contrasting sharply with the general orange background of the crater bottom. "If the correctness of the colour transmission is confirmed", says the report with some caution, "this may indicate that the bottoms of these craters are composed of rock different from that on the surface". Or (even more cautiously) "maybe, after all, there still exists vegetation on Mars?"

Passing quickly from these speculations, however, the report states a number of quantitative facts: the

The not so red planet

from Vera Rich

argon content of the Martian atmosphere (according to mass spectroscopy measurements from Mars 6) has the very high value of $35 \pm 10\%$ —a result at present inexplicable, although relative argon enrichment of the atmosphere as a result of the freezing of CO₂ to form the polar caps has been postulated tentatively.

The amount of water vapour in the Martian atmosphere was found to be much higher than measured by Mars 3 and at places the amount of precipitated water reached 60-70 μm , some 3-5 times higher than the values obtained from the Mars 3 data of 2 years ago. The distribution of this moisture over the surface of the planet was found to be irregular. The joint Franco-Soviet polarimetry experiment gave results far lower than expected, the greatest polarisation (up to 10%—some 2-3 times less than that of the Moon)—being observed close to the northern extremity of the Argyre crater sea.

Infrared measurements gave surface temperatures very close to the

theoretical models for highly fragmented rock; the highest temperature recorded being 272 K, corresponding to 1300 h local time. Correlation of surface temperature and relief has still not been observed.

Photography of the topographical features, apart from the curious problem of colour, has produced interesting results. With the high resolution used (down to tenths of a kilometre), the existence of two forms of crater was established: large craters having a flat bottom and slopes considerably affected by wind erosion, and small cup-shaped craters.

For a mission only partially successful in its hardware, the Soviet Mars survey of 1973-74 has not been unrewarding. Perhaps, however, the most significant data recorded are those from the analysis of the phase variation of the radio signals from the probes. From this it has been estimated that the electron concentration in the Martian ionosphere is 6×10^3 electrons per centimetre at night and 1.5×10^5 electrons per centimetre by day. It seems likely that on the basis of these figures, future Mars probes will use wavelengths longer than 500-1,000 m for radio communication.

THE Think Tank's recent review of energy conservation possibilities (see *Nature*, July 5) is the subject of five weekly meetings at the Royal Institution where some of the propositions and recommendations put forward in the report will be discussed and criticised. Last week's meeting dealt with the potential of tidal power—on which the report was unenthusiastic—with a quick look at wave power, regarded more favourably by the Think Tank.

The first point stressed by the speakers, Mr David Gwynn and Mr Brian Severn of Engineering and Power Development Consultants—a member of the Balfour Beattie Group—was that tidal power does work and that there is experience available in facing and overcoming the technical problems which arise.

Nobody claims that tidal power is the answer to Britain's energy problem. A scheme in the Severn Estuary, for instance, could provide a maximum of 4.5 GW and would save about 5 million tonnes coal equivalent each year in fossil fuels. But it is an inexhaustible resource and, even on the limited scale that would be possible in Britain, could produce a useful minor diversification of the energy base as part of an integrated power generating system.

There is a tide

The disadvantages of tidal power when viewed simply as an isolated source of electric power generation are to some extent mitigated when it is considered as part of a total energy system. The snags are, of course, that power generation is irregular. In any period of 25 hours there will be four periods of slack water when power cannot be generated and the range of tides also varies considerably.

The possible role of tidal power in providing a source of heat for district heating would be one way of overcoming the disadvantages of irregular power generation. Mr Gwynn and Mr Severn also see a role for tidal power in smaller local projects (not necessarily in Britain) in integrated schemes for estuary basins involving water storage, and in recreation (yachting, for example) with a small tidal power station serving some local use such as pumping water for irrigation.

One of the assumptions in the Think Tank report was that water storage and power generation in the same estuary would be incompatible. This was challenged by Mr Gwynn who pointed out that limited freshwater storage in 'bunded' reservoirs would not interfere with tidal movement. Larger schemes

for barrages across the mouth of the estuaries for water storage are now to some extent out of favour.

Compared with tidal power, the practical capabilities of wave power are as yet untried. And there are still many unsolved problems such as storing the power generated and transmitting it to the shore, quite apart from the possible technical difficulties in manufacturing the massive steel structures which are needed for the most promising scheme involving wave power.

But in the long term wave power could theoretically provide an inexhaustible source of almost all Britain's energy requirements, and is the one new 'unconventional' source of power singled out by the energy conservation report as worthy of serious investment and consideration.

Some wider implications of the use of wave power were touched on by the meeting chairman, Professor John Page of the University of Sheffield. What effects, he wondered, would the presence of strings of these massive structures out in the Atlantic have on overall wave movement and what could be the consequences to coastal ecology and fisheries? Also, in the present confusion and uncertainty over the rights of nation states over the oceans, would there also be objections on legal grounds? □

correspondence

Bacterial engineering

SIR,—The anxiety expressed by various scientists on the hazards of partially hybridising certain types of micro-organisms has already led to considerable public disagreement. It is clear that some responsible and established workers consider it unwise to take humanity with them on any tour into the unknown, while others consider it impractical to curtail the spread of organisms by any method proof against technical errors, psychoses, and even earthquakes. This will remain true whatever is stated by any of the many committees which are likely to advise each advanced nation.

I wish to advance a simple positive proposal which cannot fail to increase the safety of any bacterial incorporation studies, and which would itself provide interesting examples of extreme adaptation which might have applications to other fields, such as waste disposal.

Contracts should be offered for the development of an organism, with suitable qualities for work on partial

hybridity, which would not grow within a pH range of 6–8, would need an oxygen partial pressure of at least three-fold the normal, and which was dependent on at least two unusual synthetic substrates. These should be very simple safeguards against any single mutation allowing reproduction in any natural host.

Once such an organism was established, it should only be necessary to provide substantial subsidies to firms undertaking to provide the necessary culture media—and such media could hardly be misappropriated—to provide an economic and technical climate within which temptations to continue to work on the worst possible organism—a commensal of the human gut—would be seen as both unnecessary and irresponsible.

Since even bacteria take time, and moratoria cannot be expected to contain curiosity for long unless alternative outlets are provided, and seen to be imminent, the matter would seem urgent, and probably too urgent for the attention of the present public funding

organisations. Even if no immediate funds are available from such sources informed discussion might influence some companies which supply media and equipment for tissue culture to initiate work in the hope of reaping the large commercial rewards such developments could yield.

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Why then publish?

SIR,—Recently I was a co-author on a paper in *Biochim. biophys. Acta*. Of the 140 reprint requests addressed to me no less than 70.5% were addressed to an unknown Dr B. R. Carter. I hope this frequent oversight is not a reflection on the powers of observation of the modern scientist. Perhaps this is one way of discouraging people from publishing too many papers: deny them the right of recognition.

Yours faithfully,
B. R. CATER
Sheffield, UK

SIR,—It is regrettable that your journal should have given such prominence to an article—"Doubts Over US in India" (*Nature*, September 20)—consisting of vague allegations and innuendoes against respected scientific bodies. In the entire article I found only three factual statements capable of verification (the others being matters of opinion) and these are quoted from a letter to a newspaper. For the rest, the author seems to rely on unnamed "Indian scientists" or "experts". No names or designations are given and it is difficult to place much value on such anonymous allegations.

I would like to state the facts about the Bombay Natural History Society and its collaborative research with the World Health Organisation (WHO) and other organisations, with which I have been personally connected, so that your readers have an opportunity to form their own judgement of the matter.

In 1957 a 'new' virus disease was detected in Mysore State. This Kyasanur Forest Disease (KFD) virus closely resembled the virus of Russian Spring Summer Encephalitis and raised the possibility that the disease was brought to India from Siberia by arthropod vectors carried by migrating birds. A collaborative study of the problem was organised by the WHO, under Dr Salim Ali as principal investigator, in which the Bombay Natural History Society studied the migratory birds and the Virus Research Centre, Poona, carried out the virological studies on the birds and their parasites. After some time, it seemed that transmission of disease by this route, though possible, was not a significant epidemiological or public health problem. The WHO, accordingly, discontinued its support of the programme. The Bombay Natural History Society continued to work, although no virological studies were possible since neither the Virus Research Centre nor the Haffkine Institute (where I was then Head of the Department of Virology) could spare the necessary staff for a study. For a time, at the instance of the WHO, blood samples were sent to the Institute of Diseases with Natural Foci at Omsk, since many migrant birds come to India from that area.

At about this time the United States Army Migratory Animal Pathological Survey (MAPS) organisation was studying migratory animals and a proposal for continuing the bird migration study in collaboration with them was accepted with the approval of the Indian Ministry of Defence. No American scientist was designated for this programme by the MAPS. In addition to the migration studies, blood smears from the birds were collected and, at the outset, were sent to the

MAPS for study. Such slides could have been provided to any other agency interested. After a while the MAPS discontinued this study and several hundred slides are still at the society awaiting study by some interested party.

Subsequently grants under PL480 funds were made to the Bombay Natural History Society in collaboration with the Smithsonian Institution for a study of bird migration, and this continued until 1973 when all PL480 grants were stopped by the Indian government. These grants are made for

Answer from India

from A. N. D. Nanavati,
Bombay Natural History
Society

collaborative research programmes approved by both the United States and India, and are operated by scientists from both countries. The Indian government does not accept any such research programme without careful scrutiny by independent experts. There are hopes that the grants for this study may be revived.

The main point on which suspicion has been focussed is the collaborative study with the MAPS, because of the fear that information so obtained may be useful in biological warfare. So long as there is no secrecy involved, there can be no objection to any research, otherwise we would be constrained to ban every textbook on epidemiology and communicable diseases. This research programme was open and the information was available to anybody interested. The bird camps were visited by ornithologists and other scientists from several countries (including workers from the Institute of Diseases with Natural Foci, Omsk) all of whom had full and free discussions on the information collected. Much has also been made of the proviso that the MAPS

required that no material be published without clearance from it. There was no such provision in our contract, merely that the MAPS should receive two advance copies of any publication or report. With this information, the only factual charges made by 'a professor' and quoted by Mr Sehgal can easily be disposed of:—

(1) That blood smears were sent to the MAPS for examination. They could also have been and can still be made available to any other interested party.

(2) That two US scientists from the Smithsonian Institution were associated with the Bombay Natural History Society. One scientist, Dr Dillon Ripley was designated as the American Investigator and Dr Salim Ali was the Chief Investigator from India. This was part of the routine in any PL480 financed study.

(3) The services of the Bombay Natural History Society and the Virus Research Centre were made available to United States Army personnel for a nominal fee. This seems to be a figment of someone's imagination. Neither the Bombay Natural History Society nor the Virus Research Centre are commercial organisations. The Bombay Natural History Society offers assistance to any serious student, wherever he comes from. No charge is made for such assistance.

(4) That only partial results were available to the Indian participants. We have, in India, people of sufficient experience in epidemiology and microbiology to be able to assess the significance of these studies and to detect whether there were any significant omissions or false information in the results supplied. This charge is, however, a matter of opinion which could only be resolved if Mr Sehgal would disclose some of the information which he claims has been withheld.

It seems then that the gravamen of Mr Sehgal's charges is that all the Indian scientists working on collaborative research programmes with international or foreign agencies are gullible and will believe whatever their collaborators tell them. He therefore ignores the considered statement made by our President, Dr Salim Ali, but would place his faith in the rumours and 'feelings' of anonymous authorities, unsupported by a single verifiable factual statement. Can it be that there is no monopoly on gullibility?

This is not to suggest that we can afford to be careless. Dangers do exist and must be carefully guarded against. It is unfortunate that in trying to highlight possible dangers Mr Sehgal has cast aspersions on institutions and persons who are fully aware of their responsibility and have taken all possible safeguards against such dangers.

news and views

No consensus yet on climate

IN this issue of *Nature* (pages 368 and 370) is another instalment of the saga in which non-meteorologists suggest to the professionals processes, hitherto unrepresented, that may account for their lack of forecasting skill. The meteorologists' usual response is, as Sawyer's here, that it is not dubious new processes that are required but better understanding of those already known to be important.

Meteorologists are acutely aware that their data are so abundant that fortuitous associations of some of them with unrelated phenomena are bound to exist. To some extent King's scholarly conjectures about the association of magnetic field and climate are liable to this reservation. Sawyer shows, moreover, that there has been some selection of data in King's quoted evidence—the association he describes is much better for latitude 60° N in the winter than for other latitudes and seasons.

But I was still a little surprised at Sawyer's comment which is based on the fidelity of current numerical models of the atmosphere and in particular with his degree of confidence in what is, after all, one of the less sophisticated models of global-scale processes. To my mind this is deftly and succinctly qualified by King's reply, and one should indeed bear in mind a further qualification. The numerical models of atmospheric circulation do not represent quite such pure physics as might be supposed at first sight, for they have to be 'tuned'. By this process values of parameters appearing in the models are adjusted to make the model behave more realistically—such processes as surface friction, the transfer of energy by electromagnetic radiation and many cloud effects usually have to be treated in this way. Although this is a legitimate way of establishing a mutually consistent set of interacting processes, there is clearly room for King's as yet unidentified process.

The evidence for an association of geomagnetic and meteorological patterns presented by King is tempting (cor-

relations of 0.96 are not to be sneezed at). Causal connection is a remote possibility awaiting a plausible quantifiable hypothesis that is not even remotely hinted at by King. His speculation that "Coriolis force, inertia and viscosity of the air" may account for some differences seems naive. Meteorologists know about such things and have taken them into account in many elegant and detailed treatments which show how the atmosphere responds to any forcing, whether by anomalous heat sources, undulation of surface elevation or indeed ill-defined but energetically small magnetic effects. Such analysis shows that motion of large scale penetrates upwards high into the atmosphere, where the energy is partially reflected downwards. Such reflection is governed by the large-scale properties of the upper layers—like the general wind and temperature structure, which may indeed be conditioned by some much less intense energy sources.

Given finally that the large scale systems may be close to resonance (particularly in the winter) we have the beginnings of some process by which climate may be modified by weak energy sources, especially those in the upper atmosphere but, it must be stressed, dependent not on local anomalies of energy but on variability of the global scale circulation.

If causal effects of one on the other are unlikely, is it possible that both climate and magnetic field perturbations have a common origin? It is widely believed that the details of the geomagnetic field originate at the core-mantle boundary, if not in the core itself—an unlikely spot, one would think, for climate to be controlled from. But the obvious influence of continents on climate and the less obvious influence of continents on upper-mantle resistivity profiles could, just conceivably, be a link—the upper mantle being a window through which core effects are seen.

J. S. A. GREEN

The self control of myosin

Two recent papers have reopened the debate about the existence of a Ca^{2+} -sensitising mechanism associated with the thick filaments in vertebrate muscle.

By the late 1960s it was generally believed that contraction of vertebrate skeletal muscle—and by assumption also of other muscles—was triggered solely by the binding of Ca^{2+} ions to the troponin components of the thin filaments. The evidence for this was that troponin was apparently the only myofibrillar protein which could reversibly bind Ca^{2+} in the presence of Mg^{2+} ions and that troponin, together with tropomyosin, was required to confer Ca^{2+} sensitivity on the ATPase of vertebrate skeletal actomyosin.

The idea that all muscles were controlled through the thin filaments in this way was scotched by a series of papers by Szent-Györgyi, Kendrick-Jones and Lehman. They were able to show that whereas some invertebrate muscles had this troponin control, others (most notably certain molluscan muscles like the cross-striated adductor muscle of *Pecten*) lacked troponin but instead contained a special

kind of myosin which could bind Ca^{2+} in the presence of Mg^{2+} ions. Correspondingly, the actin-stimulated myosin ATPase from such muscles was Ca^{2+} sensitive. In other words, such muscles were controlled through the myosin of their thick filaments. Yet other invertebrate muscles had both forms of control. The test devised to determine which kind of control is present in any given muscle is simple. The effect of adding pure actin to the contractile apparatus is observed. If the Ca^{2+} sensitivity (that is the ratio of the ATPase activity in the presence and absence of Ca^{2+} ions) is reduced, then only the troponin control must be present since the thin filament activity has been swamped by the excess unregulated actin. But if the sensitivity is unaffected there must be myosin control.

Szent-Györgyi *et al.* examined both smooth and striated muscles of invertebrates but only the striated muscles of vertebrates. Now Bremel (this issue of *Nature*, page 405) has applied their test to a vertebrate smooth muscle (chicken gizzard), about whose control little is known. The

addition of pure actin has no effect on the Ca^{2+} sensitivity of a crude preparation of chicken gizzard actomyosin. This shows that a myosin control is present. To see whether or not troponin control is also present, Bremel has applied the complementary test. The addition of rabbit heavy mero-myosin to the preparation reduced the Ca^{2+} sensitivity. When due allowance is made for the activity of the chicken gizzard myosin, it turns out that the added heavy mero-myosin ATPase (stimulated of course, by the chicken gizzard actin) is not Ca^{2+} sensitive. Bremel concludes that vertebrate smooth muscle lacks troponin and is analogous to those invertebrate muscles which have only myosin-linked calcium regulation.

Even in vertebrate skeletal muscle there is growing evidence for a thick filament control. A few years ago, Haselgrove and H. E. Huxley examined by X-ray diffraction muscles stretched to lengths where no overlap would be expected between thick and thin filaments. Nevertheless on stimulation of the muscle, movements of the myosin heads, recognised by a loss of their helical order, could be observed. The simplest interpretation was that the thick filaments of vertebrate skeletal muscle were themselves directly responsive to Ca^{2+} ions. Possibly, however, some overlap of filaments was still present in local regions of the fibre and changes in the region of overlap were propagated to the rest of the thick filaments. Because there was this doubt, the significance of these important experiments was not immediately felt.

A change of thick filament conformation should affect its sedimentation coefficient. So Morimoto and Harrington (*J. molec. Biol.*, **88**, 693; 1974) have examined the rate of sedimentation of isolated natural thick filaments in the presence and absence of Ca^{2+} . A small but significant increase in the sedimentation coefficient with rise of Ca^{2+} concentration is indeed observed. The midpoint of the transition is at a Ca^{2+} concentration of about 3×10^{-6} M (well below the level of Ca^{2+} thought to be reached inside an activated muscle fibre) and is rather insensitive to the Mg^{2+} concentration. Hydrodynamic theory is unfortunately too complicated to say exactly what changes in conformation are responsible for the variation in sedimentation coefficient.

Similar experiments with synthetic thick filaments prepared from purified myosin establish that myosin, rather than C-protein, is the Ca^{2+} -sensitive protein. This was surprising since it has been assumed that vertebrate skeletal myosin binds little Ca^{2+} in the presence of concentrations of competing Mg^{2+} ions similar to that in muscle (≈ 1 mM).

Morimoto and Harrington have gone on to redetermine the Ca^{2+} binding properties of myosin in the presence of Mg^{2+} . They find that each myosin molecule has two Ca^{2+} binding sites with an affinity accounting for the sedimentation data. The sites turn out to be located on the light chains removable by 5,5' dithiobis-dinitrobenzoic acid (DTNB). It remains to be seen how the binding of Ca^{2+} ions to these chains could produce large-scale movements of the myosin heads. It is just possible that the DNTB light chains are not associated with the heads themselves (as is generally thought) but rather with the 'necks' joining the heads to the tail of the molecule. In this position they could perhaps influence the movements of the heads. The point of special interest is that the DTNB light chains of vertebrate skeletal myosin were recently shown to be capable of substituting for the Ca^{2+} -sensitising light chains of *Pecten* myosin (see *Nature*, **249**, 609; 1974) which suggests a considerable similarity between the two kinds of myosin. Nevertheless, the temptation to think of the myosin control in vertebrate skeletal muscle as being entirely analogous to that in invertebrate muscle should be resisted for the moment. There remains an important difference to explain: the actomyosin ATPase of vertebrate skeletal muscle is not sensitive to Ca^{2+} ions, whereas that of *Pecten* actomyosin is. In other words evidence is lacking that the regulation

of cross-bridge movement by Ca^{2+} affects ATP consumption. Whether this difference is attributable to a greater lack of spatial organisation of the vertebrate filaments in experiments conducted in the test tube remains to be seen. We can certainly expect a spate of papers probing this problem.

The conclusion that in vertebrate skeletal muscle there is control through the thick filaments as well as the thin filaments is attractive. It always seemed perverse of nature to control cross-bridge activity through the thin filaments when a more obvious form of control would be to regulate the movement of cross-bridges directly. The existence of two Ca^{2+} sensitising mechanisms, one on each kind of filament in vertebrate skeletal muscle, would presumably increase the sensitivity of the contractile apparatus to Ca^{2+} ion concentration and ensure that the breakdown of ATP in resting muscle was kept at a low level. The relative importance of the two forms of control remains to be assessed.

GERALD OFFER

More false hopes about solar neutrinos

ONE of the principle activities of stellar astrophysicists in the last five or six years has been to tackle the solar neutrino puzzle. Since Davis and his collaborators announced an upper bound to the flux of neutrinos incident on the earth from all sources which was only one tenth the value theorists had predicted from the Sun alone, astrophysicists have rightly been concerned about their understanding of the Sun. Initially there was a massive reappraisal of the theory, and in particular of the nuclear physics needed for computing the rates of the energy and neutrino producing reactions in the solar core. Improvements were made both to the equations governing the solar models and to the numerical methods used to solve them. But on the whole these did not change the theoretical solar neutrino flux by nearly as much as was necessary.

Perhaps the most profitable activity was to adjust certain inaccurately known parameters, such as those defining the initial conditions. This led to the construction of what has become known as the 'standard model': namely, that solar model with all such parameters adjusted to the limits of plausibility in such a direction as to minimise the predicted neutrino flux. There are now several standard models, independently computed by different workers, and all yield similar neutrino fluxes rather lower than the pre-Davis values. As to the state of the measurements, Davis has refined his techniques and now quotes an upper bound which, in round numbers, is still about a factor ten below the theoretical values.

The recognition that standard models would probably never fit the observations has provoked much questioning of the premises from which they have been constructed. Most of the papers published are of an exploratory nature, asking merely the question: "How can the theoretical neutrino flux be reduced below Davis's limit simply by arbitrarily though not entirely implausibly adjusting the unconfirmed assumptions of the theory?" A complete answer to that question would be a very valuable first step towards the solution of the puzzle.

One line of investigation has been to postulate that the inside of the Sun is rotating much faster than the surface. Early calculations along these lines were unsuccessful, but recently Demarque, Mengel and Sweigart (*Astrophys. J.*, **183**, 997; 1973; *Nature phys. Sci.*, **246**, 33; 1973) and Roxburgh (*Nature*, **248**, 209; 1974) have produced models which they thought predicted neutrino fluxes below the observed

flux bound. These models have extremely rapidly rotating cores with the ratio λ of centrifugal to gravitational forces being as high as $\frac{1}{4}$ to $\frac{1}{2}$.

But a serious flaw in the calculations has recently been pointed out by Monaghan (*Mon. Not. R. astr. Soc.*, **169**, 13P; 1974). The rapidly rotating models were computed under the assumption that λ is very small, and approximations essentially equivalent to using $1+n\alpha\lambda$ for $(1+\alpha\lambda)^n$, where α is of order unity, were used. In the calculations of the neutrino flux n is typically between 25 and 30, and for such values, Monaghan points out, the approximation leads to gross underestimates when λ is as large as Demarque *et al.* and Roxburgh require, whatever the sign of α . It seems, therefore, that even allowing for the errors arising from this approximation in the calculation of the mean energy generation rates, the quoted theoretical neutrino fluxes have been underestimated by at least a factor three.

A second criticism is raised in today's issue of *Nature*. Rood and Ulrich (page 366) have repeated the calculations

of Demarque *et al.* and have argued that the latter authors made a mistake in their computation of the oblateness of their models. This is admitted by Demarque *et al.* on page 368. When the mistake is put right, the oblateness comes out to be greater than the measured value that R. H. Dicke reports. Roxburgh's model is not ruled out by Dicke's measurements, but it is contradicted by the recently announced results of the more careful investigations by H. A. Hill and his collaborators, which imply an almost spherical Sun consistent only with a rotation of the interior hardly faster than that at the surface.

Rood and Ulrich go on to investigate the combined effect of rapid rotation and a postulated transient convective mixing triggered by shear turbulence. Of course this raises the obvious difficulty of justifying why the Sun is in a unique peculiar transient state at the present time; but in any case the models presented today fail to yield sufficiently low neutrino fluxes combined with an adequately small oblateness, even though they are based on

the small λ approximation and are subject to Monaghan's criticism. Rood and Ulrich conclude that rapid rotation is not a clue to the solution of the neutrino puzzle. They are probably right.

D. O. GOUGH

Inbreeding to preserve diversity

from our Animal Ecology Correspondent

IN the Uganda kob antelope there exists a system of social behaviour designed to prevent clandestine matings. Individual males fight for and guard small territories of between 10 and 20 m in diameter. A cluster of 30 to 40 such territories is called a lek. In the Torro Game Reserve in Western Uganda there are 18 such leks in an area of 400 km². Courtship and mating take place within the territories amid a complex and ritualised dressage which seems to be designed as if to publicise the fact that a mating has taken place. The details of the lek behaviour are well known (Buechner, *Science*, **133**, 698; 1961; *Proc 16th int. Congr. Zool.*, **3**, 59; 1963), but what is not well known is why clandestine matings should be frowned upon.

Buechner and Roth (*Am. Zool.*, **14**, 145; 1974) have investigated the possibility that the total population is divided into socially defined breeding demes, each of which occupies a lek. Most leks persist for long periods in the same place—of the 15 major leks serving the Torro Reserve now, 10 are in traditional locations which can be dated back at least to 1959. Evidence that animals breed only within a lek comes from tagging data and from translocation experiments. Most territorial males are found within 500 m of their home lek 90 to 100% of the time. Females are less attached to the site but all known individuals returned to the home lek for breeding. Until they reach 1½ years when they settle down in a lek, fawns seem to have little attachment to any particular site but on no occasion did an adult male translocated to a foreign lek succeed in establishing itself.

Morphological data, such as ear, tail and hind leg length, provide further evidence of inbreeding. There was significant variation between leks in males' hind leg and ear length, and females' body and tail length. Yet more evidence came from the animals' altruistic behaviour which, according to Hamilton (*Man and Beast: Comparative Social Behaviour*, edit. by J. F. Eisenberg and W. S. Dillon, 59 Smithsonian Institution Press, Washington; 1971) is directly related to the degree of kinship within a population.

Raindrops in the laboratory

from our Chemical Physics Correspondent

DROPS of water, and eventually rain, grow from small droplets as they fall under gravity through slightly supersaturated air. Such a system is difficult to study in the laboratory as the droplets whose growth is of interest do not remain long in the field of view of any microscope and, unlike snowflakes, the drops can not reasonably be collected and studied at leisure.

Gollub, Chabay and Flygare (*J. chem. Phys.*, **61**, 2139; 1974) have applied the special properties of the argon ion laser to this problem with great success. Basically the droplets scatter the laser light and, because of their falling motion, they impart a Doppler shift to the light frequency of the order of 500 Hz. The exact shift will depend on the velocity of fall of the droplet through the viscous air and hence on the size of the droplet which can therefore be deduced from the Doppler shift. The shift can be accurately measured by beating the scattered light with light, unshifted in frequency, scattered from the stationary walls. The effective scattering volume is only 0.02 cm³ and is well defined in location so that the droplet size can be measured as a function of position in the sample chamber. This is 3.5 cm high internally and 10 cm in diameter so that control of temperature, of air purity and of super-

saturation are straightforward to apply. Clearly different conditions are possible but in the experiments reported at 7° C and a degree of supersaturation of the order of 4%, the mean droplet radius is about 3 µm towards the top of the chamber and increases to about 7 µm as the particle falls to the bottom. At any specific point the distribution of drop sizes about the mean for that point is remarkably narrow, for example, ± 0.2 µm in 4 µm about 2 cm below the point at which nucleation occurs.

Confirmatory evidence on the particle size measurement is obtained from the scattering intensity. The growth of the droplets as a function of the distance they have fallen provides information about the growth mechanism; the results support a reasonably high thermal accommodation coefficient and even the value unity is not excluded. The condensation coefficient seems to be less than unity but doubtless further work will improve the details of the theory of drop growth. Though the results from these first experiments are important, probably even more important is the introduction of a new, informative and fairly simple and inexpensive technique which will enable the formation of water droplets and of rain to be extensively studied in the future.

The kob antelopes show altruistic behaviour in that individual males do not pursue females into neighbours' territories, victors of border disputes do not inflict serious wounds on losers, and individuals expose themselves in a conspicuous manner to predators inviting attack from a 'safe' distance. The latter behaviour has been investigated in detail by Smythe (*Am. Nat.*, **104**, 491; 1974).

Thus the courtship customs and social behaviour of each lek seem effectively to prevent matings outside the lek. Such matings are rare and their illegitimate offspring rarely survive. Social behaviour therefore maintains the genetic heterogeneity of the population by preserving the differences between leks. This ensures that there is sufficient genetic scope for evolution if conditions change, a mechanism which is probably especially important for the preservation of alleles with a small selective advantage (Wright in *The Evolution of Life*, edit. by S. Tax, 429, University of Chicago Press; 1960). A solution also exists in this successful species population to the problem of maintaining a certain amount of gene flow—every so often temporary leks are formed, lasting one to five years, after which time animals return to their original lek.

Phonon transmission

from P. V. E. McClintock

THE transmission coefficient of phonons travelling from a silicon crystal into superfluid helium exhibits a maximum when the phonon energy corresponds to a temperature of about 5 K, according to a thermal pulse experiment by Swanenburg and Wolter of the Philips Research Laboratories at Eindhoven described in a recent issue of *Physical Review Letters* (**33**, 882; 1974).

The thermal energy in a crystal at low temperatures takes the form of quantised lattice vibrations known as phonons, which behave much like a gas of free particles each travelling at the velocity of sound. At any given temperature T the average energy of the phonons present is $\sim kT$ where k is Boltzmann's constant, so that an increase of T not only causes an increase in the phonon density, but also brings about an increase of their average energy.

It has long been known that, when heat passes from one material to another, there is a discontinuity in temperature at the junction. The phenomenon can be understood in terms of an acoustic mismatch theory: just as in the case of light which undergoes partial reflection when incident on the interface between two media in which its velocity is different, so also are

phonons partially reflected if the velocity of sound differs in the two materials, thus affecting the transmission of thermal energy between them.

The velocity of sound in liquid helium is about an order of magnitude smaller than in most solids, so that there is a correspondingly large temperature discontinuity when heat passes between liquid helium and a solid, describable in terms of a thermal boundary resistance called after Kapitza, the Russian physicist who discovered the effect. For $T < 1$ K values of the Kapitza resistance measured in equilibrium heat flow experiments are in reasonable agreement with those predicted by an acoustic mismatch model. When $1 < T < 2$ K, however, the Kapitza resistance falls below the predicted values by an order of magnitude or more, an effect which is still not understood and which Swanenburg and Wolter have sought to investigate by means of their heat pulse technique.

Their experimental arrangement consisted of a very high quality silicon crystal, one face of which was in contact with liquid helium and the opposite face of which was in vacuum. A thin resistive metal film heater was deposited on the latter face. By applying an electrical pulse to the heater, high energy phonons could be generated, all of which necessarily passed into the silicon. A certain proportion of the phonons incident on the silicon-helium interface travelled on into the helium where they generated a pulse of the thermal wave mode known as second sound, which could be detected by a bolometer positioned a few millimetres away. The size of the bolometer signal was proportional to the energy flux entering the liquid. Because of the thermal discontinuity between them it was possible to heat the metal film to a very much higher temperature than that of the crystal. Thus it was possible to transmit phonons of energies equivalent to as much as 34 K into the crystal at 1.8 K, and then to investigate what proportion of these high energy phonons was transmitted into the liquid helium, which also remained at 1.8 K.

Unexpectedly, the authors found that although the transmission coefficient increased as the average phonon energy rose from 2 to 5 K, it then passed through a maximum and subsequently decreased as the energy was raised further. They describe a number of detailed checks, involving the use of heaters with different surface areas, which they have carried out in order to ensure that the change in the bolometer signal was really due to changes in the phonon transmission coefficient at the interface, and was not an artefact arising from some frequency dependent process in the crystal: they

conclude that the phenomenon is genuine.

A new theory of the Kapitza resistance is therefore required which can not only explain why the transmission of thermal energy across a solid-liquid helium interface is anomalously enhanced above 1 K, but which is also able to account for Swanenburg and Wolter's conclusion that the responsible mechanism decreases in efficiency as T is raised above 5 K.

High speed colour schlieren photography

from J. Kim Vandiver

THE cover picture of the bullet and candle is a colour schlieren photograph. It exploits techniques that were first used by August Toepler in 1864, and later by Schardin, who developed the first colour system in 1934. Though many improvements were made, until 1971 all colour methods gave only one-dimensional information about the density gradients in the test section. In 1971 a technique was described (Strong and Settles, *Scient. Am.*, **225**; May 1971) which simplified the equipment and made possible colour photographs with fully two-dimensional density gradient information. These optical refinements were combined with a sub-microsecond light source and triggering device to photograph a variety of transient events.

Refraction occurs when light encounters a region of non-uniform index of refraction. The refraction will be toward the region of increasing index in proportion to the gradient of the index perpendicular to the direction of travel. Natural events that cause changes in the pressure, density or temperature in the media around them ultimately alter the refractive index. Schlieren techniques discriminate light that has been refracted by a physical event from the remainder of the light in the test section. Once isolated, this light is focused by the camera into an image of the original disturbance.

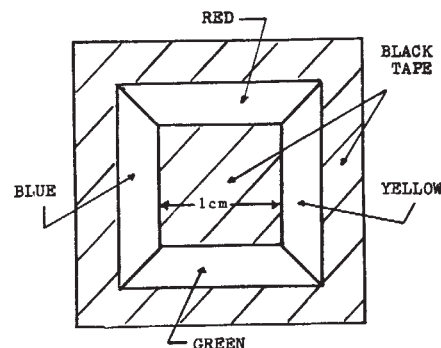


Fig. 1 Source filter

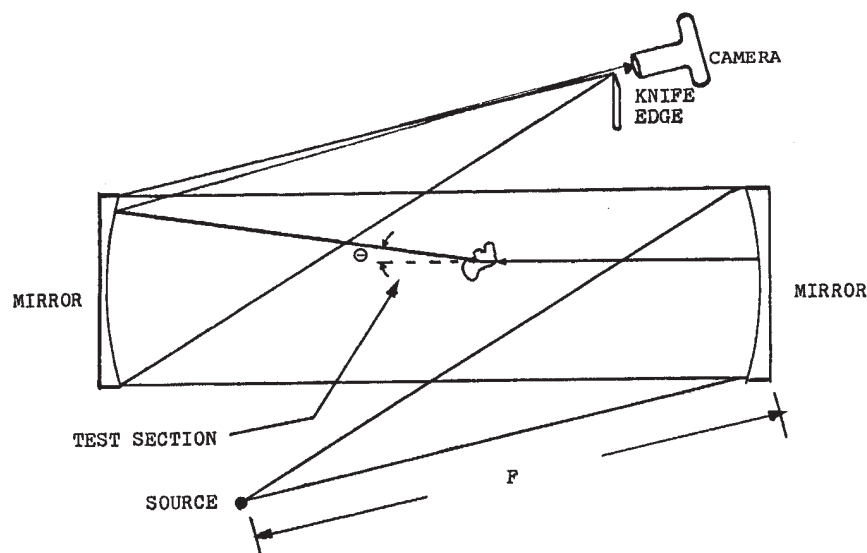


Fig. 2 Mirror system

Figure 2 depicts a simple schlieren system, consisting of two parabolic mirrors with a small light source at one focus and a knife edge and camera at the other. The test section is located in the collimated beam. The knife edge blocks the undisturbed image of the source, but lets pass light which has been refracted and deflected away from the knife edge. This yields a one-dimensional black and white photograph of the gradients in the test section.

The technique used here requires a multicoloured filter at the source, constructed as shown in Fig. 1, and four knife edges arranged to form a square aperture. The colour photograph so obtained indicates the direction of the gradient of the index of refraction by the hue of the colour in the photograph. The complete colour spectrum is used to provide 360 degrees of directional information.

Schlieren systems are very inefficient users of light. Most of the light available in the test section is rejected at the knife edges. To achieve properly exposed colour photographs requires intense light sources or long exposure times. To capture a supersonic bullet, or the shock waves from an electric spark, requires exposures of less than a microsecond. Our photographs employed an E. G. & G. Microflash Unit. This device discharges a 0.05 mF capacitor at 17 KV through a 2.5 cm air gap. Seven watt-seconds are discharged in 3×10^{-7} seconds. The light produced is focused by a reflector on to the rear of the coloured filter. Initial tests indicated that a small camera format and a fast colour film were required. A 35 mm format and High Speed Ektachrome (ASA 160) film were selected. A 400 mm telephoto lens was required to match with the 0.273 m

diameter, 2.46 m focal length parabolic mirrors, to yield an image size appropriate to the 35 mm format.

A variety of supersonic bullet photographs were taken. Bullet shock wave interaction with candle flames, soap bubbles, and solid objects was photographed revealing reflected, refracted and transmitted shock phenomena with excellent clarity. Bullet photographs were triggered, using a microphone, by the passage of the shock wave. Open air gap sparks, generating spherical shock waves, were photographed in air and water. Triggering was accomplished by a photocell and a variable time delay. Vortices are shed by propeller tips of an ordinary fan. These can be photographed when hot air from a flame is entrained by the vortex. Real time viewing with a synchronous strobe behind the filter is most revealing. More photographs may be found in Strong and Vandiver, *Scient. Am.*, 231; 105; 1974.

The simplicity and versatility of this schlieren system are assets which make colour schlieren photography an attractive tool for scientific investigation, apart from its extensive use in supersonic wind tunnels. Other features include high resolution and sensitivity and pleasing colour results.

Diseases of fodder crops

from A. J. H. Carr

FORAGE and fodder crops occupy about 75% of the agricultural area of the United Kingdom and contribute two thirds of the livestock intake. Perhaps surprisingly, the limitations imposed by disease on optimum output from these crops are only now becoming gener-

ally recognised. This was the opening theme of a symposium, "Diseases of forage and fodder crops" held at the British Museum (Natural History) on November 1 under the auspices of the Federation of British Plant Pathologists.

Introducing the topic, Dr A. J. H. Carr (Welsh Plant Breeding Station, Aberystwyth) referred to the difficulties of assessing yield losses in herbage crops which consisted of mixtures of out-breeding species grown together in competition, managed and utilised in many different ways, and with an ultimate yield assessed as animal production. Despite these difficulties, experience has revealed that infection with pathogenic fungi and viruses could cause adverse changes in botanical composition, loss in (and altered seasonal patterns of) productivity, and reduced nutritive value.

Dr A. J. Heard (Grassland Research Institute, Hurley) and E. T. Roberts (Agricultural Development Advisory Service, Reading) said that two thirds of grass crops surveyed had suffered damage due to disease and management problems in roughly equal proportions. They and others, referred to the damage to ryegrass, the predominant sown grass species, caused by crown rust, drechslera leaf spots and foot rots, and viruses such as ryegrass mosaic and barley yellow dwarf. Crown rust disease could reduce ryegrass productivity by 20% when compared with resistant varieties or plots protected by systemic fungicides and it also affected regrowth (Dr C. O'Rourke, Oak Park Research Centre, Carlow, Eire). The carbohydrate content of grasses could be reduced by half following infection with fungal leaf pathogens, and ryegrass mosaic virus reduced yield by 30% and accentuated winterkill. A valuable contribution came from F. G. Cook (Huddersfield Polytechnic) who described methods of relating production loss under various management systems to lesion development in ryegrass infected with *Drechslera* spp., which would have application to the study of other grass leaf pathogens. Dr E. G. Gray and J. F. Copeman (North of Scotland College of Agriculture) discussed the special problems arising where winter damage following extended snow cover is intensified by snow moulds (*Fusarium nivale* and *Typhula incarnata*). This influences the choice of ryegrass varieties towards those less susceptible to these pathogens. A different type of winterkill not associated with snow cover but dependent upon cold weather was described by Dr J. I. Holmes and Dr A. G. Channon (West of Scotland College of Agriculture). They showed experimentally that damage by *Fusarium nivale* was considerably increased if the inoculated grass was exposed

intermittently to low temperatures.

Dr G. R. Dixon (National Institute of Agricultural Botany, Cambridge) drew attention to the increasing problem of clover rot in white clover and discussed keys for assessing varietal resistance which existed in both red and white clovers.

The epidemiology of virus diseases, which are systemic and often transmitted both by contact and animal vectors, was the subject of papers by Drs R. W. Gibson and R. T. Plumb (Rothamsted Experimental Station, Harpenden) and by J. A'Brook (Welsh Plant Breeding Station, Aberystwyth). The Rothamsted workers, by using a filtered-air ventilated polythene tent over the crop to exclude mite vectors, were able to show that mechanical transmission played only a minor part in the spread of ryegrass mosaic virus. By contrast, the Aberystwyth work indicated that mechanical transmission was the predominant feature in the spread of the beetle-transmitted cocksfoot mottle virus. Large catches in the autumn in west Wales of aphids carrying barley yellow dwarf virus to grass and cereal crops were associated with low wind speeds, low precipitation and a prevailing southerly wind. Dr Plumb also drew attention to a number of virus problems in continental Europe which were potentially dangerous for the United Kingdom.

As the opening speaker had remarked, the solution to the problem of crop losses lay in applying the knowledge gained to the development of sound management techniques and the production of resistant varieties. Progress in resistance breeding was reported by P. W. Wilkins for crown rust and ryegrass mosaic virus and by T. D. Johnston for mildew and clubroot in swedes and rape (Welsh Plant Breeding Station, Aberystwyth). In view of past experience with pathogens of other crops it was gratifying to note that plant breeders were aware of the dangers of using simply inherited resistance, particularly to air borne pathogens, although for clubroot, caused by a soil-borne pathogen with a slower dissemination of genetic variants, such resistance could be beneficial.

Due emphasis was also laid on the nature of resistance. Mrs J. A. Chamberlain (Welsh Plant Breeding Station, Aberystwyth) presented evidence to show that tolerance in breeders' material to ryegrass mosaic virus was associated with certain ultrastructural features such as the size and number of pinwheel inclusions in host cells. Similar phenomena were associated with the tolerance of wheat to agropyron mosaic virus. J. A. Hargreaves and Dr J. W. Mansfield (University of Stirling) demonstrated the importance

of the individual components of the multiple phytoalexin response in controlling infection of broad beans by species of *Botrytis*.

Probably the most valuable lessons learnt at this symposium were the gains to be made in production from attention to management and disease problems, and the pitfalls involved in dealing with these complex biological systems.

Herpesviruses and oncogenesis

from George Klein

THE second meeting on this topic, held in Nürnberg between October 14 and 16, dealt mainly with four types of virus known or suspected to be oncogenic in man or animals: Epstein-Barr virus (EBV) in man, Marek's disease herpesvirus (MDV) in chickens, herpesvirus saimiri and atelopes in monkeys, and herpes simplex virus (HSV), defective mutants of which are thought to interact oncogenically with various animal or human target cells.

A central issue concerning EBV is the relationship of findings with animal experiments using virus-carrying cell lines *in vitro* to clinical findings in man. EBV is now widely accepted as the cause of infectious mononucleosis. Similarly, it may endow the lymphocytes with the capacity for continuous proliferation. Cells from such cultures, which carry multiple copies of the viral genome but only occasionally produce infectious virus, can grow malignantly in immunologically crippled animals such as nude mice.

Aetiological alternatives

Some workers, including W. and G. Henle (Children's Hospital, Philadelphia), Dr G. Miller (Yale University School of Medicine) and G. Klein (Karolinska Institute), believe that analogous virus-converted cell lines arise in mononucleosis patients or healthy seropositive individuals, but are held dormant, presumably by immunological means. Against this interpretation, however, A. B. Rickinson (University of Bristol) and his colleagues presented evidence from mixing the blood of acute mononucleosis patients with cord blood lymphocytes of the opposite sex. The emerging cell lines contain the sex chromosome of the added cord blood cells as frequently as that of the patient. Since continuous cell lines do not grow from uninfected cord blood cells, they infer that the cord blood cells were transformed by infectious virus released from some cell in the blood of the infected donor. Rickinson and his colleagues propose that EBV can remain latent *in vivo* in

a state like that of herpes simplex virus in the ganglia. The question remained unsettled, however, in the absence of data from mixture experiments that could have illuminated the derivation of lines from normal seropositive donors.

In Burkitt's lymphoma, EBV-genome tests on biopsy material by nucleic acid hybridisation or by EBNA (EBV-determined nuclear antigen) have led to an unexpectedly sharp distinction between the endemic disease in Africa and the sporadic cases that occur elsewhere. Whereas 97% of the African lymphomas were found to carry the genome, the relatively small number of tumours so far tested from elsewhere were uniformly genome negative. Other lymphoproliferative malignancies also proved genome negative, irrespective of the serum antibody titre against EBV.

On the other hand, it has now been shown that cells from EBV-negative lymphomas in seropositive individuals can be superinfected with EBV *in vitro*. That suggests that those tumour cells which do carry the genome do so because they are derived from a genome-carrying clone, and not as a result of having picked up the virus *in vivo*. If this is accepted, the two main aetiological alternatives can be referred to as the 'immunological' and the 'cofactor' hypotheses. According to the first, the African BL patient differs from the normal EBV-positive individual only by some deficiency of his immune surveillance. (It must be remembered that BL is a relatively rare disease, even in the high endemic regions.) According to the cofactor hypothesis, EBV-carrying cells would not yet be fully malignant in normal individuals, and some other event would be required for full malignisation. Though at present it is impossible to distinguish between these alternatives, comparison of EBV-carrying lines derived from Burkitt lymphomas, if representative for the malignant clone, and EBV-carrying lymphoblastoid cell lines derived from normal individuals are pertinent to the question. J. E. Jarvis (University of Bristol) reported the presence of a chromosome 14 associated anomaly in 8 of 10 BL derived lines, apparently identical with the marker originally described by Manolov and Manolova. The marker was not found in other EBV-carrying lines. Together with the studies of Nilsson and Pontén that suggested a difference between the morphological, growth and marker characteristics of BL and non-BL derived lines, this evidence was at least consistent with the possibility that the BL lines may represent a special type of cell.

A remarkable change has occurred with regard to the second EBV-genome

carrying human malignancy, nasopharyngeal carcinoma (NPC). This tumour is notorious for its dense lymphocytic infiltration. Since only B lymphocytes were found to carry the viral genome in previous studies *in vitro*, it has been widely assumed that it is the lymphocytes that carry EBV in NPC. H. Zur Hausen's (University of Erlangen) *in situ* hybridisation data suggest, however, that the genome is associated with the epithelial cells. Nucleic acid hybridisation data on separated epithelial and lymphoid cells corroborated this conclusion. Klein, Giovanella and Lindahl showed, furthermore, that biopsy derived, nude mouse passaged NPC lines lost the human lymphocytes but maintained the viral genome. The EBNA antigen was also present, clearly localised in the large carcinoma cells. These surprising findings will obviously reopen the whole question of the part played by EBV in the aetiology of NPC. All available studies concur in suggesting that the genome is associated with the anaplastic or poorly differentiated NPC, but not with well differentiated squamous cell carcinomas at the same or other sites. This raises important questions about the susceptibility of the normal epithelial progenitor to EBV infection and about the part played by the virus in the genesis of the tumour.

In Burkitt's lymphoma, the striking time-space clustering of the disease in the high endemic areas gives clear evidence for environmental factors. In NPC, genetic factors may be more important. It is the most common tumour in certain ethnic groups of Chinese men but is very infrequent in Western populations. In Macao and in Thailand, intermediate incidences were reported for the offspring from intermarriages between the Chinese and non-Chinese populations. In Nürnberg, Simmons presented some new evidence suggesting that NPC patients differ from controls with regard to their HL-A constitution, in line with a postulated genetic risk.

Transformation *in vitro*

In the area of EBV-induced transformation *in vitro*, there is now firm evidence that different virus strains can differ in their biological characteristics. Y. Hinuma (Kumamoto University) reported differences in the growth, morphological and marker characteristics between lines transformed by the B95-8 and WIL strains. It was not clear whether the differences were determined by the resident viral genomes or reflected a slight difference in the affinity of the two virus strains for different types of lymphoid target cells. Independent comparisons by Menezes (University of Montreal), by Miller

(Yale University) and by Klein *et al.* showed differences between the P3HR-1 and the B95-8 virus strains. The strains both induce EBNA in comparable numbers of cells, but the unique P3HR-1 virus is unable to transform cord blood or BJAB cells, instead inducing an abortive viral cycle leading to the appearance of early antigen and to irreversible cell damage. In contrast, B95-8 virus has a regularly high transforming activity but fails to induce early antigen. This might suggest that the P3HR-1 line releases a non-transforming mutant virus, but E. Kieff (University of Chicago) reported that it has about 15% more DNA than B95-8 virus.

This has led to an interesting discussion between the herpes simplex workers, E. Kieff and B. Roizman (University of Chicago) in particular, and the EBV workers. Which is the wild type virus and which is defective? Looking at it from the lytic herpesvirus field, the transforming virus tends to be regarded as defective. But 'wild' EB virus recovered from the throat washings of IM patients has excellent transforming ability. It is clear, moreover, that the P3HR-1 virus does not induce a full lytic cycle in either the cord blood cell or the BJAB target, only short, abortive and suicidal stretches. It is also puzzling that the difference between the two virus strains is only apparent in relation to these target cells while in their own home strain both viruses seem to behave similarly. Both the B95-8 and the P3HR-1 cell lines have a proliferating, non-producer stemline, with occasional activation of the cycle in comparable, small numbers of cells. This is reminiscent of the lysogenic condition. Together with other evidence, for example differences in the producer status of foetal, newborn, adult and simian lines transformed by the same virus strain, this suggests that different lymphoid cells may vary in the degree of the restrictive control they can exert on a given virus strain. On the other hand, the P3HR-1-B95-8 virus comparison clearly shows that different virus strains may differ in the degree to which they obey the restrictions imposed by a given host cell. Somatic cell hybridisation experiments indicate that at least two different host cell controls are involved, one influencing the EA producing part and the other the late (viral DNA and viral capsid antigen producing) part of the cycle. Dominance of the more permissive character in the somatic hybrids suggests that both controls are of a positive nature.

The state of the viral genome in the transformed cells is of considerable interest. Nonoyama (University of Chicago) has reaffirmed his previous conclusion that at least a large part of

The Oklo phenomenon

from Peter J. Smith

In June 1972 a team of scientists from the French Atomic Energy Commission found that uranium samples from a mine at Oklo in the Republic of Gabon were depleted in uranium-235. In the first samples studied, the ^{235}U content was only 0.003% down from the normal average natural abundance of 0.720%; but more extensive work revealed that, in some samples, ^{235}U accounted for only 0.40% of the total. Taking into account the total mass of the Oklo deposit, this meant that about 200 kg of ^{235}U had apparently 'disappeared'—enough to produce about 10^8 megawatt-hours of energy in a nuclear power station and thus to power a moderate-size town for over 100 years.

The comparison with a power station is apt, for at a conference in Paris in September 1972 the French team proposed that the missing ^{235}U had gone to fuel a natural reactor which had operated for a period of about 500,000 years some 1,800 million years ago. At that time, the ^{235}U would have been more than four times as abundant as it is now. Neutrons produced by fission would be slowed down by environmental water, thus making them more effective in maintaining a chain reaction. Ultimately, however, the natural reactor would grind to a halt as the ^{235}U burned up and as the surrounding water evaporated.

The reactor hypothesis is still unproven. But the issue may soon be resolved, for the French AEC has decided to release some samples of the Gabon uranium and its surrounding soil to the international scientific community. Some of the material has now been received by Dr S. A. Durrani of the University of Birmingham who plans to investigate the 'Oklo phenomenon' using thermoluminescence and fission track analysis techniques developed in connection with his study of lunar samples.

the viral genomes carried by non-producer cells exist in a free, plasmid-like form, although he could not exclude that some of the approximately 50 genomes could be covalently integrated with the host (Raji) cell genome. A. Adams Lindahl (Karolinska Institute) presented evidence for covalent integration, while she also confirmed that part of the genomes were free. The latter may be present in a circular form. Nonoyama also reported that he could reduce the multiple genome load of the Raji cell by cycloheximide

treatment. The state of the remaining genome has not yet been investigated.

While no restriction studies have been made on EBV as yet, Sugden (Karolinska Institute) reported the first EBV-transcription studies. He found a more extensive transcription of the viral genome (up to 30–50%) in the producer P3HR-1 line than in the non-producer Raji line, as expected. The viral RNA sequences detected in Raji cells were a subset of those found in P3RH-1 cells; they were sufficient to code for approximately 10 proteins. EBNA is the only known viral function in transformed non-producer lines.

Immunological factors

Henle stressed the curious differences in antibody patterns between different EBV-associated diseases. Burkitt lymphoma patients with ongoing disease tend to form antibodies against one particular subcomponent of the early antigen complex (R). Mononucleosis and nasopharyngeal carcinoma patients preferentially make antibodies to another component (D). Since all known strains of EBV induce both antigens approximately simultaneously, during their cycle, these differences in the host response probably reflect the way in which the various antigens becomes available to the immune system. Considerable discussion followed the report of Henle that anti-EBNA antibodies appear very late, several months after the onset of infectious mononucleosis and are frequently low or absent in Hodgkin's disease (HD). Henle attributed this to the intranuclear nature of the antigen and postulated that considerable time is required before enough EBNA positive cells have been destroyed by the immune response. In HD the response would be often deficient.

The question why lymphoma, HD and other patients with EBV-genome negative tumours sometimes develop elevated EBV-antibody titres, was approached by P. H. Levine and colleagues (National Institutes of Health), and by Johansson and colleagues (Karolinska Institute), respectively. Both dealt with the possibility that tumour-induced immunosuppression may be responsible, but in different ways. Levine found no evidence for any relationship between the lymphocytotoxicity reaction of Herberman against established lymphoma lines, and EBV titres. This reaction is mediated by non-T lymphocytes, however. Johansson found a correlation between T-cell depression, as judged by PHA, Con A, and PPD lymphocyte stimulation or skin tests, and increased anti-EBV (VCA) titres. No such correlation was found with reactivity to a B cell mitogen (PWM). The notion that T-cell suppression may be crucial receives

support also from the demonstration by Aiuti that two congenitally T-cell deficient children had significantly elevated anti-EBV (VCA and EA) titres that fell to normal after thymus grafting and appearance of T cells in the circulation. It is likely that T cells play some regulatory part in maintaining the anti-EBV titre at a relatively low level from year to year in normal individuals. Depending on one's viewpoint, this could be attributed to T-cell mediated

Sabin withdraws claim

THE persistent irreproducibility and frustration to which Zur Hausen has alluded (see Klein on this page) is not confined to attempts to demonstrate herpesvirus DNA or antigens in cervical carcinomas. Albert Sabin has been obliged to withdraw his claim made early last year that a nonvirion antigen of HSV is associated with a large number of human cancers and can be found early in infection of guinea-pig cells *in vitro* (see *Nature*, **247**, 334; 1974).

The history of frustration has extended over a period of five months during which Sabin has been trying to trace the reasons for his failure to reproduce the results he and Tarro reported last year (*Proc. natn. Acad. Sci. U.S.A.*, **70**, 1032–1036 and 3225–3239; 1973). The irreproducibility of the results remains unexplained and Sabin's most recent paper (*Proc. natn. Acad. Sci. U.S.A.*, **71**, 3248–3252, 1974) contains a detailed admission of defeat.

The difficulty in which Sabin now finds himself weakens the case for HSV as the aetiological agent of certain cancers; hopes of shedding light on oncogenesis through a study of the function of early HSV proteins are correspondingly dimmed.

surveillance against dormant, potentially neoplastic cells, or to some T-cell dependent antibody, acting either against dormant cells or against the spreading of periodically reactivated virus.

Marek's disease and herpes simplex

An important development in the field of Marek's disease is the establishment by Kato of three permanent lines which Nazerian has shown carry 70–80 genomes per cell. IUDR can activate antigen production in a small proportion of the cells. The lines had T-lymphocyte characteristics, confirming the suspicion that the disease is due to malignant T-cell proliferation. P. M. Biggs (Houghton Poultry Research Station) showed that bursectomy did not increase the incidence of the malignant lesion. Powell found that the

established lines can serve as targets for *in vitro* lymphocytotoxicity tests. Preliminary evidence suggests that MDV-induced tumour cells may have a distinctive membrane antigen: clearly better antigen definition and immunological characterisation are urgently needed in the MD system. The disease seems to be uniquely suitable for studies on immune surveillance against tumours induced by a naturally occurring virus, with genetic differences in susceptibility and a corresponding contrast between self-limiting and malignant lesions. Biggs summarised the remarkable success story of vaccination against the malignant form, first with attenuated MDV, and later with a partially crossreactive but non-pathogenic turkey herpesvirus (HTV). It is interesting that without protecting birds from infection or reducing virus shedding the vaccine was nevertheless capable of preventing the development of malignant disease. It is probable, although unproven, that this is due to immunisation against tumour-associated antigens. The situation may be analogous to the successful protection of hamsters inoculated with polyoma or SV40 virus when newborn, by a second virus dose.

There is now increasing evidence that the HSV-transformed lines carry viral genomes. Frankel pointed out that viral RNA is often more easily detectable than viral DNA. Summers reported the detection of viral DNA using restriction fragments as the hybridisation probe.

Though laboratory models of HSV transformation have achieved a status of increased reproducibility, this cannot be said about the attempts to demonstrate herpesviral genomes or their antigenic footsteps in human cervical carcinomas. Zur Hausen said that this search has resulted in "persistent irreproducibility and persistent frustration, rather than persistent infection". This was countered by the argument that fractional genomes may be very difficult to demonstrate, as also evident from the *in vitro* transformed cell studies. The epidemiological evidence that links HSV type 2 infections to cervical carcinoma is circumstantial at best. While some careful studies suggest a relationship, at least in certain geographical areas and certain socioeconomic groups, the suspicion still prevails that the venereally transmitted type 2 infection may be another co-variable of promiscuity, particularly the early onset of sexual life and multiple partners, factors that are known to have a role in the genesis of cervical carcinoma, rather than being an aetiological factor in itself. But recent epidemiological studies of the Melnick group where controls and cancer patients have been matched with regard to sexual history speak against this.

review article

Mutational pressure as the main cause of molecular evolution and polymorphism

Tomoko Ohta*

The advent of detailed studies on the evolution of individual molecules has cast into doubt some of the neo-Darwinian concepts of what determines evolutionary change. Much more emphasis must now be placed on the constraints imposed by the structural and functional requirements of protein molecules, and on random fixation of very slightly deleterious as well as selectively neutral mutations.

As more data accumulate on evolutionary change at the molecular level, it becomes increasingly necessary to re-examine evolutionary theories¹⁻⁴, and in particular the orthodox neo-Darwinian view that the rate and direction of evolution are determined almost exclusively by positive natural selection³⁻⁵. It is now clear that at the molecular level, evolutionary change results from the spreading of all 'tolerable' mutations in a population. But much of the theoretical argument depends on the definition of a tolerable mutation. Biochemists and non-population geneticists may equate the designation⁶⁻⁹ with non-lethal, whereas to many population geneticists it means advantageous. There is, in fact, no clear distinction between tolerable and non-tolerable mutations: mutations occur continuously in any biological population, with a wide range of effects across the whole spectrum from lethal to advantageous.

Much recent evidence points to a continuum at one end of which are very nearly neutral mutations with undetectable phenotypic consequences, and at the other end of which are the mutations with a distinct phenotypic effect, and on which Darwinian selective pressures can therefore act. This point of view forms the basis for the present theory which can explain many of the recent data both on the rate of evolution as derived from studies of protein structure, and on the distribution of protein polymorphisms detectable as electrophoretic variants. Theoretically, it is possible to calculate the probability of fixation, or more generally the probability of mutants reaching a certain frequency within a population in terms of the "selection coefficient"¹⁰. If a mutant allele is selectively equivalent (neutral) to its wild-type allele, then the rate of mutant substitution in the population is equal to the mutation rate¹¹. If the mutant is advantageous, the substitution rate is higher than the rate of mutational input and *vice versa*. Thus, tolerable mutations should include all those mutations which have a finite chance of survival, and the rate of evolution is determined by the average "selection coefficient" of new mutations^{12,13}.

This can be seen in the rate of evolution in various parts of the genome. It is now generally accepted that the less functionally rigid a molecule is the more rapidly it evolves^{5,6,9,14-16}. For example, fibrinopeptides A and B and the middle segment of proinsulin are the fastest evolving molecules so far described and are enzymatically removed and discarded from the functional residue of the molecule⁵. The total genome DNA evolves at almost the same rate as these molecules^{17,18}, which agrees with the proposal that the majority of genome DNA is not

informational in higher organisms¹⁵. This idea can be extended to the high variability of the "spacer" DNA of ribosomal genes¹⁹. Yet the evolutionary rate of fibrinopeptides may be slightly less than the mutation rate. This is because some structural constraint may affect polypeptide folding²⁰ so that the rate of evolutionary substitution of amino acids in fibrinopeptides is likely to be somewhat less than the intrinsic mutation rate. It is probable that molecular evolution proceeds essentially by mutational pressure rather than by positive Darwinian selection. More and more evidence is accumulating that the maximum observed evolutionary rate is close to the intrinsic mutation rate⁵. If positive selection is the cause of such rapid evolutionary change, one must assume that, at any single period of evolutionary time, only a very few, specific, amino acid sites of a protein molecule can be replaced by definitely advantageous mutations. The remaining sites (constituting the majority) cannot accept mutations, since the fixation probability of definitely advantageous mutations can typically exceed 100 or 1,000 times that of neutral mutations in a large population. It is difficult to imagine that continuously changing environmental factors are so simple as to distinguish a few particular advantageous mutations among numerous others. Molecules are evolving individually, at a rate determined by the average selection coefficient of new mutations, which reflects the structural or functional constraints of each molecule. This view is essentially an extension of the neutral mutation-random drift hypothesis of Kimura¹ and King and Jukes², and takes into account the idea of "frozen accidents" proposed by Crick²¹ and Ohno²². Once the structure and function of a molecule are determined in the course of evolution, natural selection acts mainly to maintain them, because all later evolutionary changes proceed under selective constraints. Natural selection then becomes mostly 'negative' and positive Darwinian selection is only a minor part of both total selection and the total number of mutant substitutions. If selection pressure is very weak, a mutant allele, even if slightly deleterious, can occasionally replace the original allele by random drift, particularly in small populations, and such chance events are likely to be important in molecular evolution^{23,24}.

Higher order structures

Let us now discuss the effect of natural selection on secondary or tertiary structure, as natural selection acts through these higher order structures and not on primary structure. Probably the simplest example is the clover leaf structure of transfer RNA. I have suggested before that the coupled base substitutions in the paired region of this molecule, in the course

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of evolution, represent a very slightly deleterious base substitution in the first stage followed by a slightly advantageous complementary base substitution²³. This hypothesis is supported by the observation of Holmquist *et al.*²⁵. They have shown that G·U or U·G pairs account for about two-thirds of all non-Watson-Crick pairs in the helical regions of 43 sequenced tRNAs. They considered that since the presence of a single G·U pair does not interfere with helicity, it is an acceptable transitional stage. Other pairs such as A·C or G·A would cause greater distortion and therefore a mutation leading to such a change is less likely to spread in the population, and this non-random distribution of base pairs seems to fit the present hypothesis.

Transfer RNA is evolving at about the same rate as cytochrome *c* (ref. 25) and perhaps there is no fundamental difference between the evolutionary mechanisms of these two molecules. The molecular organisation of the secondary and tertiary structure of proteins is likely to be restored if another mutation is added after an amino acid is replaced in a well-organised system²⁰. This is considered to be the basis of the observed linkage of amino acid substitutions.

This corresponds to Fitch's concomitantly variable codons (covarions)¹⁴. Wyckoff²⁶ by comparing bovine and rat RNases found that out of 18 changes which are involved in charge differences, about 14 are paired with respect to charge. Because of highly specialised intramolecular interactions²⁷, the effect of the linkage of substitutions is apparently not the summation of the effect of each substitution. From these considerations, it is difficult to assess the adaptive significance, in ecological terms, of individual substitutions.

Selection pressure

Selection pressure must, in general, be very weak and indefinite for mutations that can only be detected at the molecular level, compared with mutations that have a visible effect. As neo-Darwinism is based on observations at the visible phenotypic level, it should be re-examined.

Consider a very small selective force as the lower limit of stronger selection. The majority of new visible mutations are deleterious, and the majority of "nearly neutral" mutations is likely to be very slightly deleterious, especially if the proteins and biochemical pathways are very highly organised and if environmental factors cannot interact directly with the primary structure of proteins. For such very slightly deleterious mutations, the chance of spreading by random drift is much higher in a small population than in a large population. In fact, if the product of the effective population number and the selection coefficient is less than unity, such mutations behave as if neutral and can spread through the population by random drift¹⁰. The product may be regarded as the number of effectively selected individuals per generation. This increases linearly with the increase of the total population size if the selective force is kept constant over a wide range whereas if the selective force is not constant, such a simple relationship may not hold²⁸. As the product becomes larger, natural selection is more effective and very slightly deleterious mutations cannot spread²³. Thus, the present hypothesis differs from the original neutral mutation-random drift hypothesis in emphasising a very small selection pressure. In fact, it is an extended form of neutral theory, conceived as the lower limit of the selective process.

Through extensive studies using *Drosophila melanogaster*, Mukai has shown that the mutation rate of viability polygenes is about 0.3 per genome per generation—much higher than previously thought^{29,30}. This rate applies to those mutations which have effects strong enough to be detected by Mukai's viability test. If there is continuity of mutational change from disadvantageous to neutral, one would expect substantial numbers of nearly neutral mutations. Let us tentatively estimate the informational genome size of *Drosophila* from Mukai's

result. If the intrinsic mutation rate per base pair per generation is roughly 10^{-8} as previously estimated^{5,15,31}, then viability polygenes should correspond to 3×10^7 base pairs, and this amounts to one-third of the genome DNA. Furthermore, if the total gene number in *Drosophila* is about 6,000 (ref. 32), the average gene size becomes 5,000 base pairs. Genes may include much of the regulatory DNA and the class of mutations whose effects are not large enough to be detected by Mukai's test. The latter class probably includes important candidates for "tolerable mutations". In my view, there should be no distinct borderline between deleterious and neutral, between deleterious and advantageous, or even between deleterious and overdominant mutations. Random genetic drift and mutational pressure play a much bigger part than has been believed.

Molecular polymorphisms

Mutational pressure is also important for the maintenance of molecular polymorphisms. Electrophoretically detectable alleles show a remarkably uniform distribution over a very wide geographical range in *Drosophila*³³. This observation has been explained by the neutralists assuming migration³⁴⁻³⁶, whereas selectionists attribute it to some forms of "balancing selection"^{33,37}. Very recently, by combining heat-denaturation studies with electrophoresis, Bernstein *et al.*³⁸ have found that each electrophoretic allele can be divided into a few heat sensitive alleles at the xanthin dehydrogenase locus in *Drosophila*. Their data, although not yet conclusive because of small sample sizes, suggest that these minor components within an electrophoretic class are geographically differentiated. They argue that electrophoretic alleles are maintained by balancing selection while heat-sensitive alleles may be selectively neutral. It is hard to imagine, however, particularly in the light of recent knowledge of protein structure and function²⁷, that natural selection makes a clear-cut distinction between these two classes. It is more likely that genetic variabilities detected by electrophoresis in large populations like those of *Drosophila* represent a mutation-selection balance. For minor components (heat-sensitive alleles within each electrophoretic class), stochastic effects due to random drift are more prominent because of lower mutational input, and hence they may be geographically differentiated. Of course urgent investigation is needed to find out if the pattern observed by Bernstein *et al.* represents a general phenomenon.

So far, observations on protein variations in natural populations have been based mainly on protein electrophoresis³⁹ which only detects mutations which express themselves as charge differences on the molecule. To analyse such variations, therefore, we proposed a model of stepwise production of alleles in which the entire allelic states are expressed by integers, and mutations are represented by moving each allele one step either in the positive or in the negative direction on the allele space^{5,40}. On the basis of this mutational scheme, we have investigated the theoretical consequences of weak negative selection (T. O. and M. Kimura, unpublished). In a typical example, we assume one type allele and multiple slightly deleterious mutations as follows:

mutation	...	$v/2$	$v/2$	$v/2$	$v/2$	...
allele	...	A_{-2}	$\rightleftharpoons A_{-1}$	$\rightleftharpoons A_0$	$\rightleftharpoons A_1$	$\rightleftharpoons A_2$...
fitness	...	$1-s$	$1-s$	1	$1-s$	$1-s$...

where v is the mutation rate per gamete per generation and s is the selection coefficient assuming genetic selection. Under this model, it can be shown that the mutation-selection balance predominates if the product of effective population size (N_e) and mutation rate (v) is sufficiently large provided that $s > v$. The distribution is determined by the ratio s/v and the results conform quite well to the observed pattern of allelic distribution, that is, the commonest allele is surrounded by less common or rare alleles. Now, if population size becomes small so that $N_e s$ is smaller than unity, very slightly deleterious alleles

become effectively neutral and the heterozygosity is determined by $N_e v$ (ref. 41).

We have already suggested from a comparison of actual observations with the results of our simulation experiments assuming stepwise production of neutral alleles, that there are more rare alleles than predicted from the strictly neutral hypothesis⁴¹. If we bring very slight negative selection into the model, the discrepancy between the observation and the simulation should disappear completely. Perhaps the recent analysis of Yamazaki and Maruyama⁴² needs re-examination in the light of this. From data on protein polymorphisms, they found that the observed frequency of heterozygosity as a function of gene frequency follows a roughly uniform distribution in the range 0.0–0.5 (because of folding back the distribution around 0.5 (ref. 43)). They used data on several hundred electrophoretic alleles and because the observed distribution agrees with Maruyama's theoretical prediction⁴³, concluded that their analysis supports the selective neutrality of protein polymorphisms. If one uses the model of stepwise production of alleles by mutation, however, the distribution of the frequency of heterozygosity is not uniform unless $N_e v$ is extremely small, even if alleles are neutral. Through an extensive Monte Carlo experiment assuming the step-allele model and selective neutrality, I have found that the frequency of heterozygosity gradually increases as the gene frequency class moves from 0.0 to 0.5 for the actually interesting range of $N_e v$ (0.05–0.5). The observations on isozyme polymorphisms thus indicate once again the existence of more sporadic alleles than predicted by strict neutrality.

Uniformity across species

A further argument against Darwinian selection as the sole determinant either of evolution or of polymorphism can be made on the basis of the remarkable uniformity of evolutionary rate and of heterozygosity between species. Individual cistrons seem to evolve at a very similar rate in diverse lines of organisms^{6,7,11,14,16}. The existence of some local variations in evolutionary rate in closely related species¹² does not materially affect the validity of this general statement^{3,5,44,45}. If the majority of amino acid substitutions is due to positive Darwinian selection in response to environmental changes, one has to assume that such changes occur at roughly the same rate in various different lineages. It is hard to believe, for example, that the carp and the human lineages have experienced similar rates of environmental change. Similar arguments apply to the uniformity of the average heterozygosity per locus in different species. While this uniformity does not follow from the neutral mutations hypothesis, which predicts an indefinite increase in heterozygosity with increasing population size, it fulfils the expectations of selectionist hypothesis no better. *Escherichia coli*, for example, has an immense population size by comparison with *Drosophila* or man, and an entirely different ecology, but approximately the same degree of 'heterozygosity' in terms of effective number of alleles⁴⁶.

Approximate uniformity in the rate of evolution is to be expected if evolutionary change proceeds essentially by mutational pressure. The uniformity in heterozygosity between species can most plausibly be explained in terms of a balance between mutational and selective pressures. In a large population, heterozygosity is close to the maximum that can result from mutational pressure balanced against selection based on the structural and functional constraints of the molecules themselves. The constraints imposed by the structural and functional requirements of the peptides are taken as almost independent of ecological conditions²⁰. The importance of such structural constraints is illustrated by the observation⁴⁷ that there is less heterozygosity for glycolytic enzymes than for other proteins, since the glycolytic enzymes might be expected to have the more rigid functional requirements⁴⁸. If similar

distinctions are not seen in some mammalian species⁴⁹, this may be because they have not yet arrived at a mutation–selection equilibrium.

Enzyme differentiation and speciation

The patterns of enzyme differentiation between sibling species in the *Drosophila willistoni* group⁵⁰ can most readily be explained by assuming that they have reached a mutation–selection balance. The patterns of allelic variation in this group fall into two classes: (i) sibling species with the same set of alleles in similar frequencies; (ii) sibling species with non-overlapping allele distributions. For loci belonging to the first category, the same mutation–selection scheme applies to both sibling species, whereas for those in the second category, I suggest that the balance has shifted at the molecular level in the course of speciation. In a well adapted species, a constant input of very slightly deleterious mutations does not result in the replacement of the original allele if the population is very large. In small populations, which may often occur at the time of speciation, very slightly deleterious mutations behave as effectively neutral and may replace the original allele by chance. Then molecular constraints will be shifted, leading to a new mutation–selection balance. Thus, the degree of neutrality of new mutations is crucial to my theory of what determines the rate of evolutionary change and speciation. If the borderline cases between slightly deleterious and completely neutral mutations are important, as I assume here, then the population size becomes most crucial and the rate of evolution is higher in small populations such as may occur at the time of speciation. It is suggestive that the amino acid substitutions seem to be concentrated at the branch points in the phylogenetic trees of cytochrome *c* and the haemoglobins^{51–53}. This tendency, however, may be a statistical artefact resulting from the assumption of maximum parsimony which leads to the postulation of hidden mutational substitutions. The true pattern of molecular evolution and variation can only emerge from further investigation, bearing in mind particularly the importance of intramolecular organisation which derives from the polypeptide folding mechanism. It is this that determines molecular function and hence is subject to natural selection, and here ecological conditions are likely to be much less significant than has traditionally been supposed. In the light of such considerations, the original concept of the integrated gene pool⁵⁴ may have to be modified.

Finally, the distinction between the strict neutral theory and my theory should be clarified. (i) The neutral theory classifies new mutations into discrete classes; deleterious, neutral and advantageous classes. In my theory, there is no clear-cut distinction among these classes and the borderline cases are assumed to be more important. (ii) The rate of amino acid substitution is higher in small populations in my theory, whereas it is independent of population size in the strict neutral theory. Consequently, my theory predicts that substitutions will be concentrated at the time of speciation, when small population size creates a "bottle neck". (iii) In very large and stable populations, my theory gives an upper limit for heterozygosity.

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articles

Sequence analysis of immunoglobulin light chain messenger RNA

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RNA and DNA sequencing methods are used to define some features, principally at the 3' terminus, of the structure of immunoglobulin light chain mRNA. The results show that there is one molecule coding for the V and C regions of the light chain and rule out the possibility of two separate molecules.

A DETAILED knowledge of the structure of messenger RNA is required for a full understanding of its function, to define not only its coding properties but other less well known properties, such as control signalling and transport. For this, the sequence of those parts of the mRNA not coding for protein—the untranslated regions—is of special importance. Until recently, technical difficulties have confined such studies to bacteriophages. But a start has now been made on the structure of the eukaryotic mRNA coding for immunoglobulin¹ and globin^{2–5}. The structure of the immunoglobulin mRNA is particularly important because of its relevance to basic problems of immunology. Light and heavy Ig chains contain a variable region (V region) which defines the antibody specificity and a region which is

constant for each type and class of chain (C region). It is believed that the chains are coded by separate pools of V and C genes and that each chain is the result of the expression of a single gene from each pool⁶. To determine the number of genes involved in each pool by hybridisation techniques, a knowledge of the structure and purity of the mRNA is required. In addition the critical question of whether the integration is achieved at the DNA, RNA or protein level has been the subject of considerable speculation. A number of experiments indicated that the chains were synthesised from a single mRNA molecule rather than from two^{7–9}. Objections, however, have been raised and direct structural evidence is essential to settle finally the argument.

In this paper we present further sequence studies on the light chain mRNA of the mouse myeloma, MOPC 21. We describe the sequence and location of the larger T₁ RNase oligonucleotides within the mRNA, and the sequence of 52 nucleotides adjacent to the poly (A). These are included within a region of about 200 untranslated bases in the 3' terminal position. Overall the results establish the presence of a single molecule involved in the synthesis of both V and C regions.

Characterisation and location of larger T₁ end products

Methods for purifying light chain mRNA have been developed by ourselves^{1,10} and others¹¹⁻¹³ for a number of different mouse myelomas. The purity achieved is difficult to assess because of the different criteria used by different workers. We have consistently used cells which have been grown in tissue culture because they represent a much better starting material than the

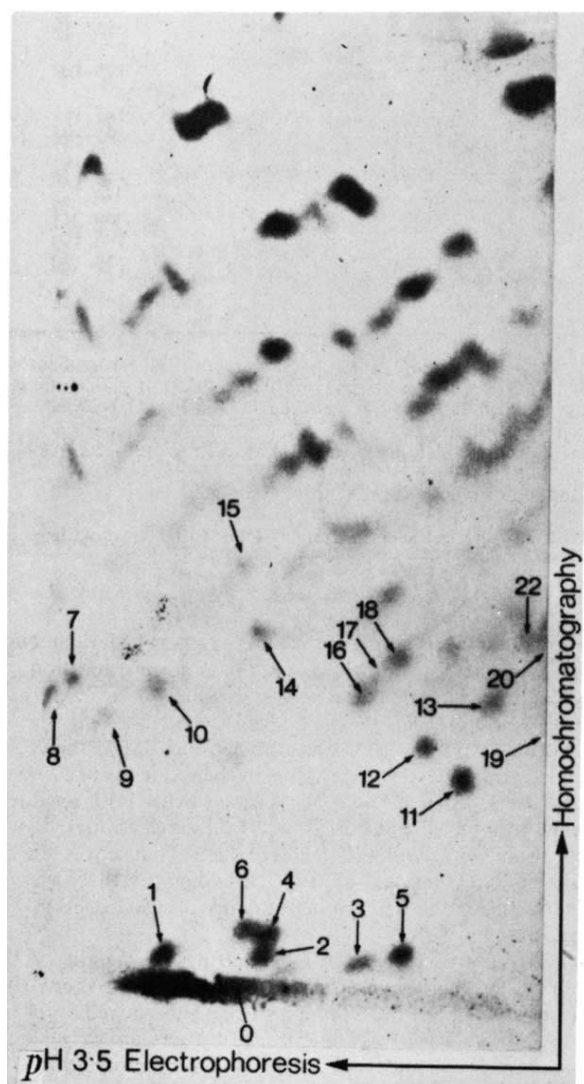


Fig. 1 Two-dimensional fractionation of a T₁-RNase digest of ³²P-labelled light chain mRNA, purified from microsomes by successive sucrose gradient centrifugation and dT-cellulose chromatography¹ and finally by acrylamide gel electrophoresis in 8 M urea¹⁰. Fractionation in direction 1 is by ionophoresis at pH 3.5 in 7 M urea using cellulose acetate ('Cellogel'); direction 2 is by homochromatography on DEAE-cellulose thin layers (1:7) using a 3%, 10 min hydrolysed nucleic acid mixture. The numbered spots were eluted and analysed by further digestion with pancreatic RNase. We were unable to use these fingerprints for more detailed sequence studies as the radioactivity in the oligonucleotides was insufficient. To prepare oligonucleotides with enough radioactivity for further analysis, a less pure preparation had to be used in which the last step of purification was omitted. Fingerprints of the 30–50% pure mRNA were more complex but all the spots numbered in the figure could be clearly identified. They were eluted and analysed by further digestion with pancreatic RNase and separately by digestion with U₂-RNase¹⁸. Spot 21 is not shown here but runs just to the right of, and midway between, spots 19 and 20 (see Fig. 3). Spot 0 is unresolved from the streak at the origin (poly(A)) here. It was resolved in other experiments by using a stronger homomixture (5% unhydrolysed) in the second dimension.

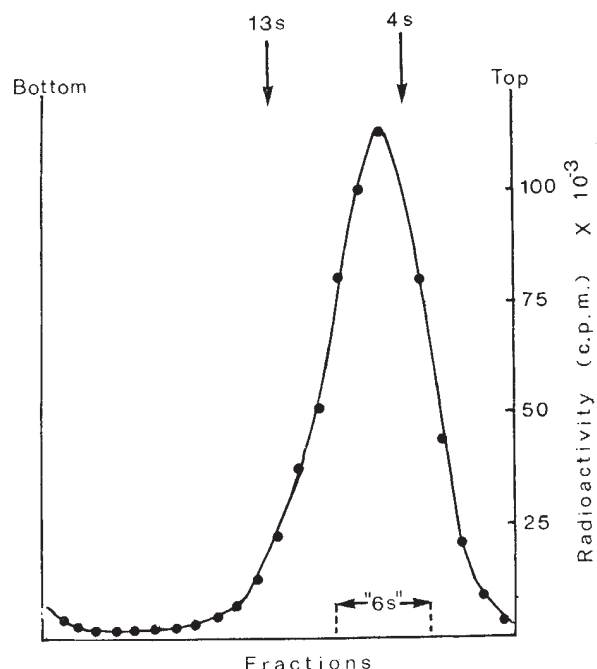


Fig. 2 Sucrose density gradient centrifugation of partially hydrolysed light chain mRNA. About 1 μ Ci of ³²P-labelled mRNA (30–50% pure material) was incubated for 25 min at 40° C in the presence of 50 mM Na₂CO₃ and 40 μ g yeast RNA. After addition of 3% SDS, 10 mM Tris Cl (pH 7.0) and 5 mM EDTA and incubation for 5 min at 70° C the fragmented RNA was fractionated in 15–30% sucrose density gradients in 100 mM NaCl, 5 mM EDTA, 10 mM Tris Cl (pH 7.6) and 0.5% SDS in the MSE 6 $\times$ 14 ml swinging rotor (35,000 r.p.m. for 17 h at 30° C). A peak of radioactivity (6S) was pooled and fractionated by dT-cellulose chromatography. The poly(A)-containing fraction (20% of the total) was precipitated with ethanol and subjected to fingerprint analysis (see Fig. 3). 4S is the position of an internal unlabelled marker of *E. coli* tRNA and 13S that of intact (unhydrolysed) mRNA run in a parallel gradient.

more generally used solid tumours grown subcutaneously in mice. Furthermore, the tissue-cultured cells are ideal for the preparation of ³²P-labelled mRNA, which is then suitable for purity studies by chemical characterisation. Chemical methods are a necessary complement to purity determination based on less reliable procedures such as homogeneity on separation systems or specific translation in heterologous cell-free systems.

In a previous report¹ we described the preparation of purified light chain mRNA. Four oligonucleotides, ranging in size from 19–22 bases in length, were isolated from partially purified light chain mRNA and aligned with specific regions of the amino acid sequence of the protein. These four oligonucleotides—and others, described below—were prominent spots in T₁-ribonuclease fingerprints, but were superimposed over an obvious background of spots presumed to derive from contaminating mRNA. We were then able to use these assigned oligonucleotides as chemical markers for the presence of light chain mRNA. Further purification was achieved by acrylamide gel electrophoresis where four bands were resolved. The light chain mRNA was identified as the only band containing the marker oligonucleotides in T₁-ribonuclease digests. Evidence of purification was also provided by the much lower level of background oligonucleotides in the fingerprint.

Figure 1 is an example of a fingerprint of light chain mRNA purified by acrylamide gel electrophoresis showing the marker oligonucleotides (spots 2–5), with only a very faint background of contaminating material, and many other products in yields equimolar¹⁰ to the markers, as would be expected from a pure molecule of the size of 13S. These additional oligonucleotides must, like the markers, derive from the mRNA and all those

Table 1 Alignment or non-alignment of T₁ end-products with coding region of mRNA

Product (Fig. 1)	Probable sequence or partial sequence*	Alignment† (V, C or X)	Light chain (residue nos of amino acids)
0	(A ₃ -C, A-C, C ₂₆ , U ₁₂)G	X	
1§	(A-U ₂ , C ₉ , U ₅) A ₂ -G	X (3)	
2§	U-A-U-C-C-A-U-C-U-U-C-C-C-A-C-C-A-U-C-C-A-G	C	115-122
3§	A-C-C-C-A-A-U-C-U-U-C-C-C-A-A-A-U-C-C-A-U-G	V	5- 11
4§	A-C-A-U-C-A-A-C-U-U-C-A-C-C-C-A-U-U-G	C	200-206
5§	A-A-C-A-A-C-U-U-C-U-A-C-C-C-C-A-A-A-G	C	137-143
6	(A ₄ -U, A ₂ -U, A-U, C ₃ , U ₂) A ₃ -G	X (3)	
7	(A-U ₂ , C ₂ , U ₄) G	X (3)	
8‡	C-U-A-A-U-A-U-U-U-G	X (3)	
9	A-U-U-U-C-A-C(U, C) U-G	V	70- 73
10	A-U-U-A-U-C-A-C-U-G	V	85- 88
11a	(C ₅₋₆ , U ₂) A-C-A-A-G	X (3)	
11b‡	A-A-A-U-A-A-A-A-C-G	Switch	105-108
12a & b¶	(A-C ₃ , C ₂₋₃ , U ₂) G	X, X (3)	
13	A-C-A-U-A-A-C-A-G	C	188-191
14	C-U-A-U-A-C-C-U-G	C	191-194
15	U-C-A-C-C-U-U-G	V	19- 21
16	A-C-A-U-C-A-A-U-G	C	143-146
17	U-A-U-C-A-A-C-A-G	V	36- 38
18	C-U-U-C-A-A-C-A-G	C	208-211
19	C-C-A-C-U-C-A-C-A-A-G	C	196-199
20	C-A-C-C-C-U-C-A-C-G	C	177-180
21	(C ₄ , A-C ₂) A-G	X (3)	
22	C-A-C-C-U-A-C-A-G	C	171-174

* Sequences were derived from results of both the pancreatic RNase and the U₂-RNase end products of each spot¹ and alignment with a predicted sequence derived from the known amino acid sequence. Where only partial sequences are shown, the products of pancreatic RNase digestion are listed. In these cases the quantitative values are only approximate. Two oligonucleotides (not marked on Fig. 1), both between 10 and 15 bases long, were not included in the table because of incomplete sequence information.

† V, C and X show the alignment of the oligonucleotide with either the variable (V), constant (C) or neither (X) region of the mRNA. (3) shows that the nucleotide was in the 3' untranslated region (see Fig. 3).

‡ The sequence of 8 and 11 b was deduced solely from the sequence analysis and independently of the protein sequence.

§ Previously characterised¹.

¶ Two oligonucleotides are present as a mixture and may be distinguished by their different 3' ends after U₂-RNase digestion. At least one is assigned with the 3' untranslated region.

longer than nine residues, as well as some smaller ones, have now been studied (Table 1). Of these, 16 (including the four previously characterised) are assigned to the coding region—five corresponding to the V region, 10 to the C region, and one (11 b) to the link or 'switch' between V and C. This sequence corresponds to residues 105-108, as follows:

105 108
Glu Ile Lys Arg
(G)AA AUA AAA CG

These are residues that are generally considered to be in the V region, except for the arginine which could be described as the first of the C region. Nine oligonucleotides (Table 1) did not fit with any of the sequences predicted by the protein sequence and should therefore belong to the untranslated region. If so, they should be located either at the 5' or the 3' end of the molecule. To investigate this problem we introduced a small number of random breaks in the intact mRNA by means of mild alkaline digestion. Then, the fragments of a given size range were fractionated by oligo (dT)-cellulose chromatography (Fig. 2). In this way, the segments of mRNA which remained attached to the poly(A) (3' end of the mRNA) could be investigated by fingerprinting. The relative simplicity of this fingerprint (Fig. 3) showed that the fractionation was specific, and the presence of poly(A) that it derived from the 3' end. A further indication of the simplicity of the fingerprint was the separation observed in the region of the smaller oligonucleotides (top of the fingerprint). Thus spots 51 and 52 were resolved from the background, whereas in the intact mRNA they were unresolved from other components of related sequence. The numbered oligonucleotides (Fig. 3) were characterised by digestion with pancreatic ribonuclease. Almost all of the nucleotides assigned to the untranslated region (1, 6, 7, 8, 11a, 12a and 21, but not spot 0, and possibly one of the components of 12, see Table 1) were present in the fingerprint, but the oligonucleotides assigned to the coding region (for example 2, 3 and 5) were absent or present only in very low yields (see below). Thus all the main components are located between the coding region and the poly(A).

The above results suggest that there is a considerable number of bases in the 3' untranslated region. A simple addition of the lengths of the oligonucleotides 1, 6, 7, 8, 11a, 12a, 21, 51 and 52 gives a value of 106-108 residues. This excludes all the oligonucleotides shorter than hexanucleotides and therefore is obviously an underestimate. Thus in the sequence of 52 residues immediately adjacent to poly(A) (see below) 13 residues in digests of T₁ RNase occur as small oligonucleotides. If we include these we account for 120 residues. That leaves the other small oligonucleotides of Fig. 3, which at a conservative estimate make the minimum total up to 150 bases in the 3' untranslated region.

An alternative method for estimating the length of the 3' untranslated region was based on the measurement of the molecular weight of the 6S preparation. This ranged evenly from 0.7×10^6 to 1.7×10^6 , as determined by acrylamide gel electrophoresis in formamide¹. We calculated, therefore, that the 6S fraction is a mixture of fragments varying in size from 220 to 530 bases. In a population of molecules such as this the relative yields of oligonucleotides in a T₁ ribonuclease digest will be inversely proportional to their distance from the poly(A) at the 3' end. An examination of Fig. 3 shows that most of the spots in the coding region of the mRNA are completely absent, although three spots, 18, 19, and 4, are present in low yield. These correspond to residues 208-211, 196-199 and 200-206 respectively of the light chain. The largest fragments of the 6S fraction, therefore, include about 60-80 bases corresponding to the last 20-25 amino acids of the light chain. Subtracting this figure from the longest fragments in the preparation (530), we calculate that the 3' untranslated region is about 450 bases long, including the poly(A). Assuming that the poly(A) is still 200 bases long (in these longest fragments), the length of the 3' untranslated region is about 250 bases. This length measurement is critically dependent on the accuracy of the molecular weight determination and on our ability to recognise marker oligonucleotides. Spots present in less than 10% yield of the major spots on the fingerprint of Fig. 3 are probably not detected.

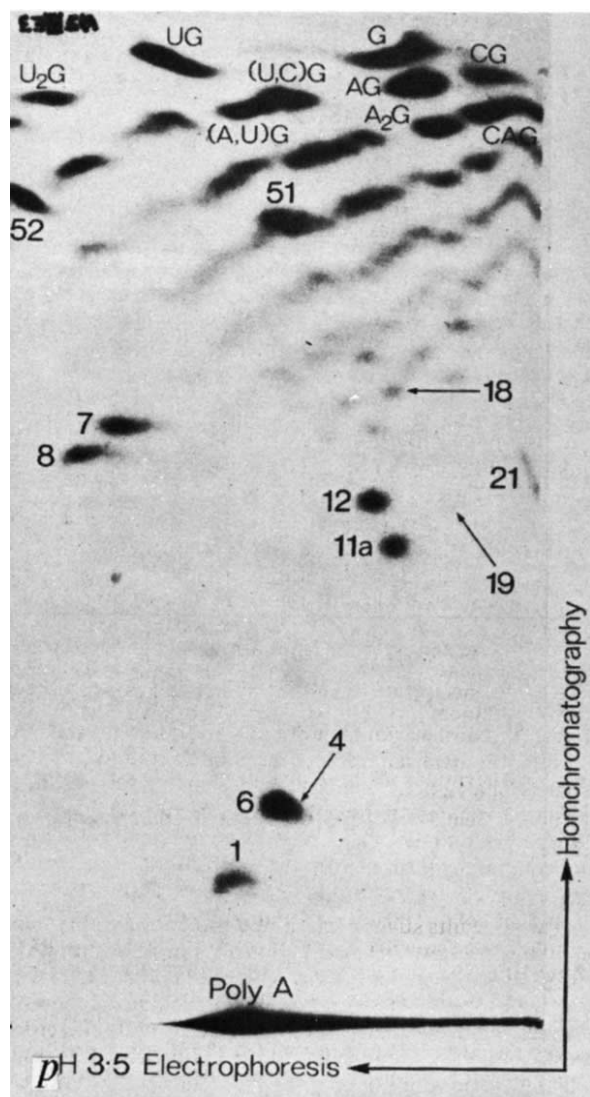


Fig. 3 Two-dimensional fractionation of a T_1 -RNase digest of the poly(A)-containing 6S fraction isolated by partial alkaline hydrolysis (Fig. 2). Fractionation was as in Fig. 1 except that a stronger 'homomixture' (5% unhydrolysed) was used for the second dimension. Spots were numbered from their position by comparison with Fig. 1, eluted and their identity confirmed by further digestion with pancreatic RNase. Spots 4, 18 and 19 were in too low yield for this check to be made. Spots 51 and 52 are relatively small oligonucleotides not isolated in intact mRNA. Their pancreatic products indicated that they were (A-C, U₂, C) G and (U₄, C) G respectively. The structure deduced for other smaller spots is shown directly.

This would give an overestimate of the length of the 3' untranslated region and 250 bases is likely to be a maximum value.

Despite the discrepancy in the values obtained (150 and 250 residues) we have argued that the lower value is an absolute minimum and the upper value probably a maximum value. Taking an average, we conclude that there are 200 ± 50 bases present in this region.

Sequence of 52 bases adjacent to poly(A)

An alternative approach to sequencing the 3' end of the mRNA involves the use of the reverse transcriptase activity of DNA polymerase I from *Escherichia coli*^{2,5}. With an oligo(dT)₁₂ as primer, synthesis of complementary ³²P-labelled DNA (cDNA) starts at the poly(A) or 3' end of the mRNA. Thus (³²P) CTP-labelled cDNA was prepared using light chain mRNA

digested with endonuclease IV and fractionated (Fig. 4). The fingerprint obtained was highly characteristic (see legend to Fig. 4). The seven oligonucleotides numbered 1-7 (and two other spots—8 and 9—which were obtained from [α -³²P] GTP-labelled cDNA) were sequenced using the techniques of partial exonuclease digestion, depurination and nearest neighbour analysis^{15,16}, to give the DNA sequence shown in Fig. 5. A comparison of this sequence with the results discussed in the previous section shows that it is complementary to some of the larger T_1 oligonucleotides shown to be in the 3' untranslated region of the mRNA (spots 6, 8, 51 and 52 of Table 1). Thus we conclude that DNA polymerase must be synthesising an accurate complementary sequence. The mRNA sequence may be drawn in the form of a double hairpin loop by maximising the extent of base pairing. The resultant structure is shown in Fig. 5a of the accompanying article⁵, where a comparison with a related region in rabbit β -globin mRNA is presented and discussed.

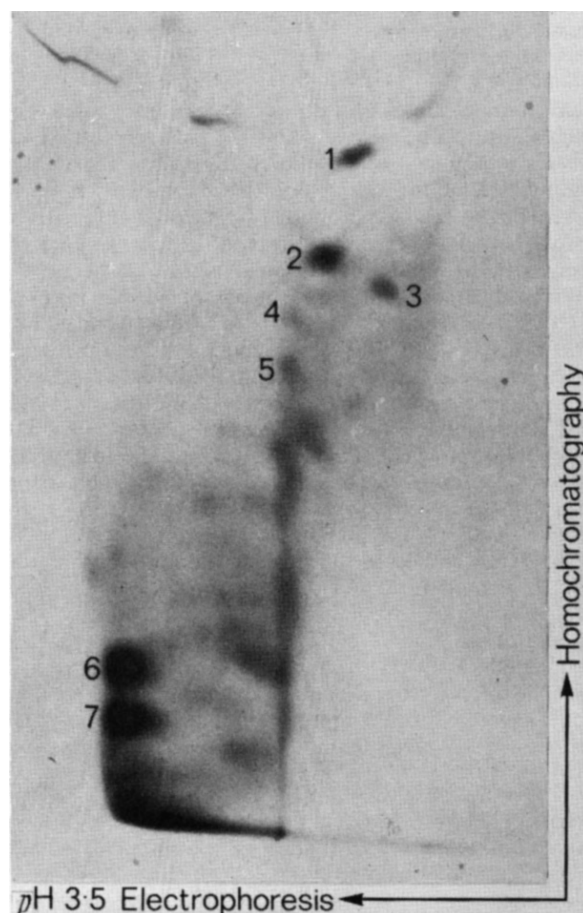


Fig. 4 Two-dimensional fingerprint of an endonuclease IV digest of (³²P) dCTP transcript of immunoglobulin light chain mRNA. The mRNA preparation was contaminated with DNA. Therefore it was heated to 100°C for 5 min and digested with pancreatic deoxyribonuclease (0.2 mg ml⁻¹) in 10 mM Tris chloride pH 7.5 and 10 mM MgCl₂ at 37°C for 30 min. EDTA was then added to 20 mM, the solution taken to 95°C and mRNA extracted using a phenol chloroform mixture (50:50) at pH 9.0. The RNA was concentrated by ethanol precipitation and transcribed using the Klenow subfragment of DNA polymerase I (*E. coli*) and Mn²⁺ with [α -³²P] dCTP (specific activity 100 Ci mmol⁻¹), and dATP, dGTP and TTP. The cDNA was isolated and then subjected to endonuclease IV digestion as described in the accompanying article⁵. Spots 5 and 6 were more prominent in other experiments. The unmarked prominent components near the origin of the second dimension are due to incomplete digestion by the endonuclease IV. In addition there is a complex background of weaker spots. Some are known to represent minor digestion products, while others are uncharacterised but may be due to longer cDNA copies or to transcripts of contaminating mRNA or DNA.

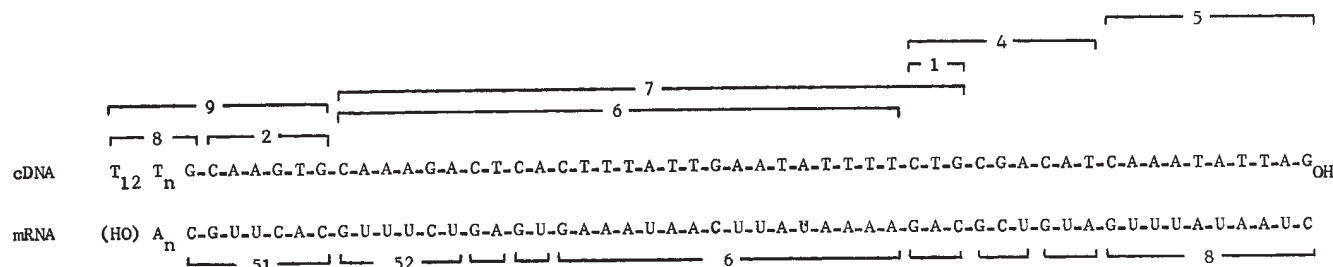


Fig. 5 Sequence of cDNA transcript of mRNA. The numbers above the sequence refer to the products of endonuclease IV digestion shown in Fig. 4. The positions and sequences of the complementary T₁ oligonucleotides (Fig. 3 and Table 1) to this sequence are as shown below the sequence. It should be noted that there is no formal overlap between the endonuclease IV spots 2 and 6 on the one hand and 4 and 5 on the other hand. The arrangement shown was established by the kinetics of labelling of the cDNA.

Implications of the structural studies

A unique sequence of 52 bases has been established for the 3' end of the mRNA immediately adjacent to the poly(A). This is included within a region of 200 (± 50) bases located between the coding region and the poly(A). In addition, a catalogue of 25 oligonucleotides ranging from eight to about 45 bases long is presented, and accounts for about 345 residues or one third of the length of the mRNA (excluding the poly(A)). Of these, 194 bases have been assigned to the coding region accounting for 75 amino acids out of a total of 214 of the light chain. From these results we can now propose a simple outline diagram (Fig. 6a) for the mRNA defining the approximate lengths of the various regions. Of particular interest are the 3' and 5' untranslated regions (3' and 5' UT in Fig. 6). The length of the 3' UT (200 bases) is probably accurate to within 50 bases, but that of the 5' UT region (150 bases) is much less accurate as it was derived by difference and is subject to the errors in all the other determinations¹. A knowledge of the extent of the 3' UT region is critical to experiments in which complementary DNA is used for hybridisation with cellular DNA, designed to determine the number of V and C genes¹⁷. The function of such a long 3' untranslated region remains unknown.

Figure 6a shows the presence of V and C regions within a single mRNA molecule. An alternative model in which the V and C regions occur on separate molecules—illustrated in Fig. 6b—deserves special consideration in view of the existence of separate V and C genes. In both models we have used a length of 1,250 bases for all molecules because this is the size of the mRNA from which oligonucleotide markers for both regions have been isolated (see Table 1). Model *b* predicts that there should be two separate sequences for the two 3' UT

regions. We have only found one such region—the 52 residue long sequence shown in Fig. 5. Nevertheless, it could be argued that the two mRNA molecules depicted in Fig. 6b have identical 3' untranslated sequences. If this were so, the yield of oligonucleotides derived from the two regions should be twice that of those nucleotides present in either the V or the C region. The relative yields of some of the large T₁ oligonucleotides of Table 1 have been measured¹⁰ and are as follows: Spot 1 = 1.0; spot 2 = 1.3; spot 3 = 1.0; spot 4 = 1.1; spot 5 = 1.2; spot 6 = 0.8. It is clear that the yields of spots 1 and 6, which derive from the 3' UT region, are equimolar within experimental error with those of various markers in the coding region (spots 2–5). Thus, the data contradicts the model. In addition, model *b* predicts that there are over twice as many residues in untranslated regions of the mRNA as in the coding regions. Table 1 shows that 194 bases have been assigned to the coding region compared with 118 in the untranslated region. These figures again contradict model *b*, but lend support to model *a*. A 'switch' oligonucleotide spanning residues 105–108 of the amino acid sequence (spot 11b of Table 1) was isolated. Although this overlap of the V and C regions is rather too short to be considered as independent evidence for a single mRNA molecule it is fully consistent with it. Thus the structural studies reported in this paper confirm the hypothesis that there is a single mRNA molecule containing the sequence information for both the V and C regions of the light chain protein.

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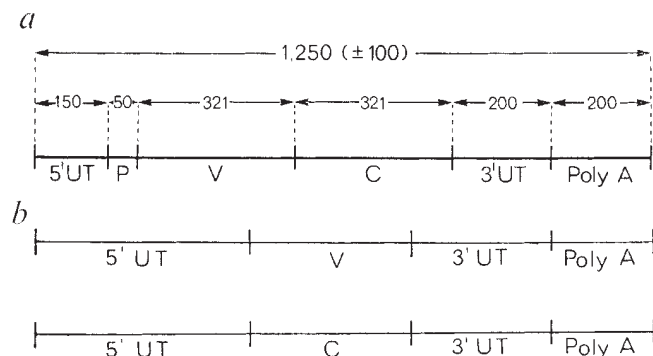


Fig. 6 Diagrammatic representation of the MOPC 21 light chain mRNA. *a*, Arrangement of the various regions and their length in numbers of bases. The lengths of the V (variable) and C (constant) regions are derived from a knowledge of the protein structure. The length of the 3' untranslated region (3' UT) is discussed in the text while that of the poly(A) and of the total mRNA is taken from ref. 1. P: precursor region¹⁹. *b*, Hypothetical alternative arrangement of V (variable) and C (constant) regions on two different mRNA molecules (see text).

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Sequence at the 3' end of globin mRNA shows homology with immunoglobulin light chain mRNA

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The sequence of 52 nucleotides adjacent to the poly(A) of rabbit β globin mRNA has been determined. Striking sequence and structural homologies exist between it and mouse immunoglobulin light chain mRNA. These homologies may be common to all mammalian mRNA.

The messenger RNA (mRNA) coding for globin is an obvious choice for detailed sequence determination. It is easily purified and can be obtained in good yield^{1,2}. All the mRNAs that have been purified including globin mRNA, are known to contain more nucleotides than are required for coding³. These extra residues, presumably present on each side of the coding region, and defined as the 5' and 3' untranslated regions, are of unknown function. They must, however, include control elements, presumably defined by nucleotide sequence. Functions such as translational control, the termination of transcription, the addition of poly(A), the binding of transport proteins and mRNA degradation are likely candidates.

Although globin mRNA has been available in a purified form for several years, the determination of its sequence has not been seriously attempted. RNA sequencing by radioactive techniques has been successfully applied to transfer, ribosomal and viral RNA species, but has not been extensively used for mRNA because of its size and the difficulty of labelling it *in vivo*³. We report here the use of a new approach that overcomes these difficulties. Instead of labelling the mRNA strand *in vivo*, a radioactive DNA copy is synthesised by means of an RNA-dependent DNA polymerase. Reverse transcriptase, from various sources is well known to be active in this synthesis, but we have used, in preference, the reverse transcriptase activity of DNA polymerase I of *E. coli* which is very active in the presence of low concentrations of manganese⁴. An advantage in using a DNA polymerase is that synthesis starts only from a double stranded region. Such a region is defined at the extreme 3' end of the mRNA by hybridisation of oligo dT to the poly(A). Also, recent work in this laboratory^{6,8} has established elegant procedures for the sequence analysis of radioactive DNA.

Globin mRNA sequence

Radioactive complementary DNA (cDNA) was prepared using each [α -³²P]-labelled deoxynucleoside triphosphate of high specific activity in turn and the other three unlabelled triphosphates, with DNA polymerase and manganese. The conditions of incubation were identical to those previously described⁴ except that a subtilisin fragment of DNA polymerase I was used⁵, which lacks the 5' exonuclease activity of the enzyme. In these conditions short cDNA transcripts, about 50 nucleotides in length, were synthesised. The ³²P-cDNA was purified and digested with endonuclease IV (refs 6 and 7)

followed by two-dimensional fractionation (Fig. 1). The oligonucleotides thus separated were sequenced using the techniques of partial exonuclease digestion, depurination and nearest neighbour analysis^{6,8}.

A detailed account of this work will be presented elsewhere, but as an example of the techniques that were used the fractionation of a partial venom exonuclease digest of spot 9 is shown in Fig. 2. The sequence of this oligonucleotide was tentatively deduced from the relative mobility of the spots⁸ giving the sequence as shown in the figure. Partial digestion with both venom and spleen exonucleases were particularly useful techniques. Once a successful digest and fractionation was obtained, as in Fig. 2, it was relatively straightforward to confirm the postulated sequence by using the standard methods of depurination and nearest neighbour analysis, with each of the four input labels. In this way, all the endonuclease IV products, except the very large ones close to the origin of the second dimension, were uniquely sequenced.

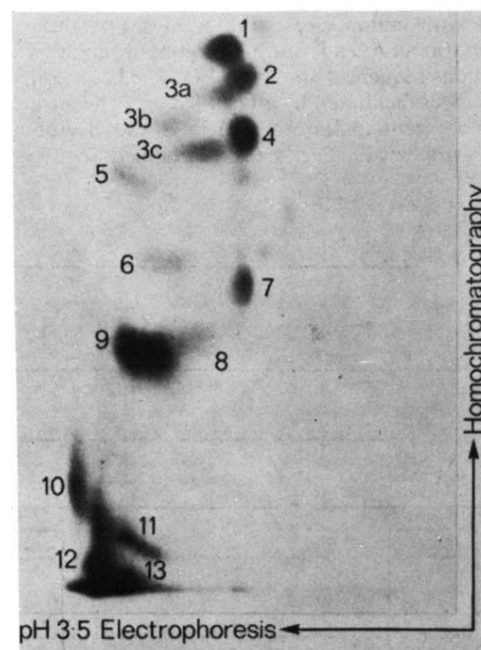


Fig. 1 Two-dimensional fingerprint²² of an endonuclease IV digest of the cDNA of rabbit globin mRNA. [α -³²P]-GTP-labelled cDNA⁴ was purified by pancreatic digestion (10 μ g ml⁻¹ pancreatic ribonuclease at 37°C, 30 min) followed by phenol extraction with a phenol-chloroform-isoamyl alcohol mixture (50:50:1) several times. The aqueous layer was ethanol precipitated, acid precipitated and dissolved in 30% triethylamine carbonate pH 10.0. Finally the triethylamine carbonate was removed by freeze drying with distilled water washes. The sequences of the spots are identified in Fig. 3.

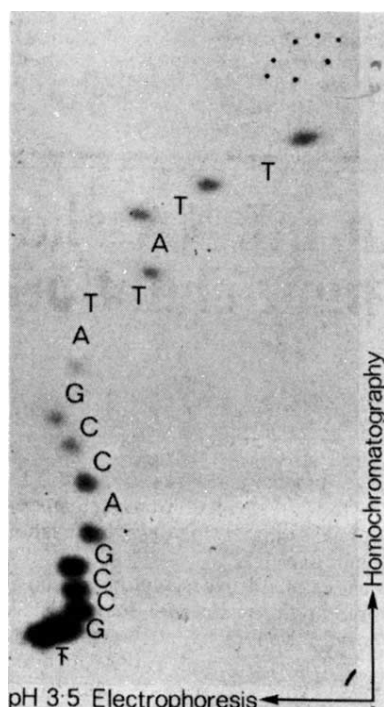


Fig. 2 Two-dimensional fingerprint²² of a partial venom exonuclease digest⁹ of spot 9. The sequence (5')--T-T-A-T-T-A-G-C-C-A-G-C-C-G-T (3') was deduced from the relative mobilities of the intermediates and confirmed in further experiments.

The final sequence was obtained by overlapping the sequenced oligonucleotides. This proved to be relatively easy, as amongst the oligonucleotides of Fig. 1 were larger products of partial digestion with endonuclease IV. Analysis of these products using depurination and nearest neighbour analysis gave the unambiguous sequences shown in Fig. 3. The overlap with the oligo dT₁₂ was facilitated by a knowledge of the tetranucleotide sequence adjacent to the poly(A) in α and β globin mRNA previously reported⁴. The main overlapped sequence derives

from the higher yield oligonucleotides in Fig. 1. Some lower yield products, however, notably 5, 6 and 7, are unaccounted for in this sequence.

A problem in sequencing globin mRNA is that, as usually isolated, it is a mixture of both α and β mRNA, as may be shown by translation experiments in heterologous cell free systems. From the sequence results described above it seemed likely that our particular preparation of globin mRNA, by contrast, contained predominantly one of the mRNA species (either α or β globin mRNA) and relatively little of the other. To establish the identity of the major sequence, the mRNA preparation was assayed by translation in a wheat germ extract⁹. Characterisation of the ³⁵S-methionine-labelled product by the analysis of the labelled tryptic peptides¹⁰ showed that 5–10 times as much β globin as α globin was synthesised. We conclude that our mRNA preparation contains predominantly β globin mRNA and that this is the source of the major (52) sequence. Although the minor sequence of the α globin is incomplete, homology between the α oligonucleotides and the β mRNA sequence is apparent (Fig. 3).

Hairpin-loop structures

Figure 4 shows the β globin mRNA sequence (complementary to the β globin cDNA) drawn in the form of two possible hairpin-loop structures, maximising the extent of base pairing. The stability of the hairpin loops may be assessed¹¹ and the longer hairpin loop (labelled II in Fig. 4) has a large negative free energy (ΔG (25° C) = -10 kcalorie), suggesting that it is a stable entity. The smaller hairpin loop has either zero free energy (Ia) or a value of +4 kcalorie (in Ib), suggesting that it is thermodynamically unstable.

In spite of this, we suggest that a double hairpin-loop structure exists and that it is stabilised by interactions with other parts of the mRNA or with specific proteins. Of course, we admit that although plausible, such a secondary structure for the 3' end of globin mRNA may be totally incorrect, and that proof of this or any other arrangement must await the results of direct physicochemical studies. Whether or not the α mRNA sequence has the type of secondary structure shown in Fig. 4 is also of great interest, but awaits further study. Considerable homology does exist, however, between the two mRNA sequences as would be predicted for two such functionally related molecules.

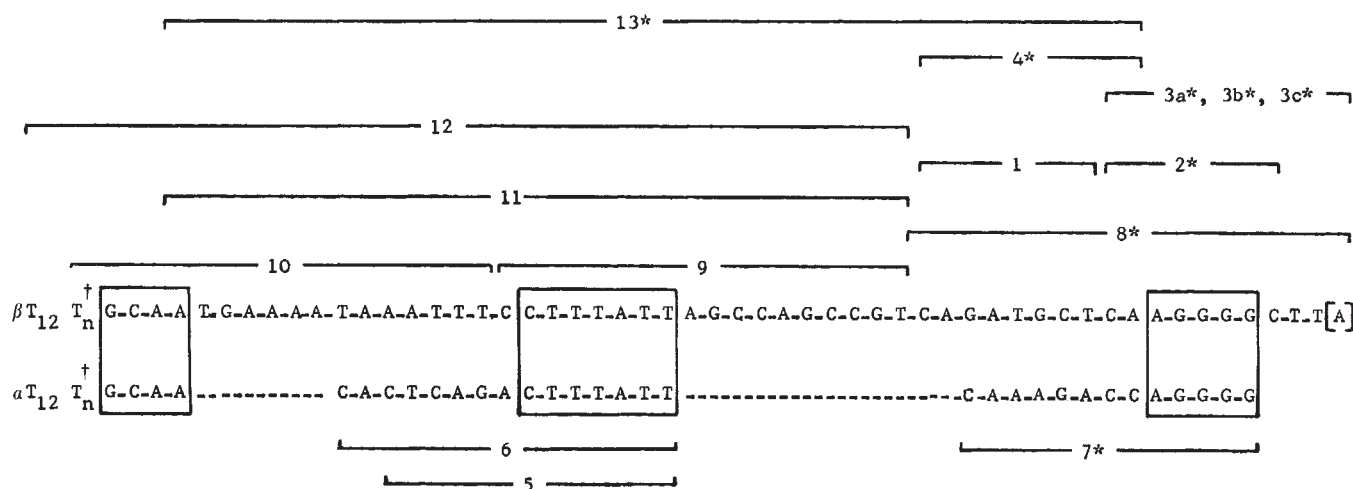


Fig. 3 Summary of sequence data on the oligonucleotides numbered in Fig. 1. The sequence for the cDNA of the β globin mRNA is fully overlapped. The oligonucleotides derived from the cDNA of α globin mRNA are ordered by homology with the β sequence. Homology between the two sequences is denoted by the boxes.

* The oligonucleotide's 3' end is a result of termination of transcription.

† n varies depending on the position that the oligo dT₁₂ primer hybridises to poly(A). The square brackets denote a sequence ambiguity.

The amino acid sequence of rabbit β globin is known¹² so that, using the genetic code, it is possible to partially predict the mRNA sequence corresponding to the carboxyl terminus of the protein but the sequence reported here shows no homology with this region and it is clear that it is entirely within the untranslated region. A lower limit of 52 nucleotides for the length of the 3' untranslated region is therefore established. Our recent work on longer cDNA copies of β globin mRNA has made it possible that the 3' untranslated region is substantially longer than 52 residues. Molecular weight determinations of mouse and rabbit β globin mRNA have suggested that β globin mRNA contains about 200 untranslated nucleotides^{13,14} (excluding the poly(A)), although it is not known how these are distributed between the 5' and 3' untranslated regions. Moreover, it can be predicted from the studies on chain termination mutants¹⁵ of human α globin mRNA that at least 96 residues are present in the 3' untranslated region. Our results are consistent with these studies.

Globin and immunoglobulin mRNA sequences

The sequence of 52 nucleotides adjacent to poly(A) in the mRNA coding for the light chain of an immunoglobulin mRNA has been determined by similar methods to those described here and is described in an accompanying article¹⁶. In this case, it was demonstrated that DNA polymerase in the presence of manganese, copies the mRNA faithfully. Thus, we have no reason to suspect that the sequence reported here has any errors due to mistakes in transcription.

Figure 5 shows a comparison of the β globin and immunoglobulin light chain mRNA sequences. A striking sequence and structural homology exists between these two mRNAs, if the globin mRNA sequence is drawn in the 'b' form of Fig. 4. The homology between the two mRNAs may be considered in two parts, sequence and structure. Both possess the sequences A-A-U-A-A-A-G and U-U-G-C-poly(A) (enclosed within the boxed area of Fig. 5). These two sequences are also present

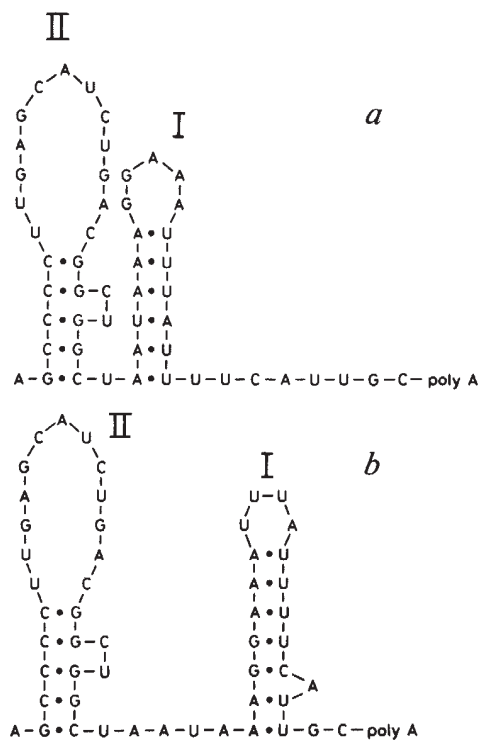


Fig. 4 Two possible conformations for the β globin mRNA sequence drawn in such a way as to maximise base pairing. I and II, hairpin loops whose estimated stability is discussed in the text.

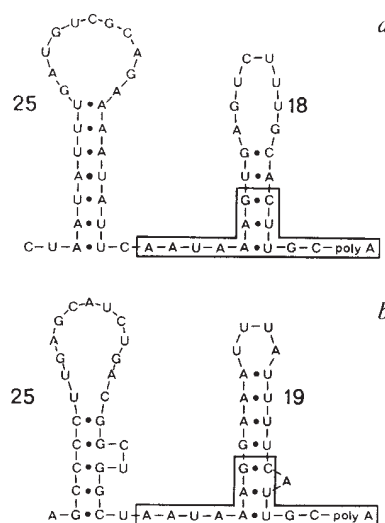


Fig. 5 Comparison of the 3' end of, a, mouse immunoglobulin light chain mRNA with that of, b, rabbit β globin mRNA. Sequence in boxes denotes homology between two mRNAs. Number adjacent to each hairpin loop denotes the total number of nucleotides in that loop.

in α globin mRNA (Fig. 3). A double hairpin-loop structure can be drawn for both β globin and immunoglobulin light chain mRNA (Fig. 5). The loops are identically positioned with respect to each other and are almost the same size in the two mRNAs. The sequence homologies mentioned above are in both cases at the base of the smaller loop and are again identically positioned. Much of the homologous sequence is therefore probably in an accessible unpaired region.

The striking similarity, both in sequence and in secondary structure between globin and immunoglobulin mRNA suggests that this untranslated region of the two molecules has a common function. One part of this sequence, that immediately adjacent to the poly(A) (U-U-G-C) may well be recognised by poly(A) polymerase¹⁷. This nuclear enzyme is thought to be responsible for the post-transcriptional addition of poly(A) to mRNA precursors¹⁸ (HnRNA). *In vitro* studies have shown however, that this enzyme has no requirement for a specific sequence¹⁹. This apparent contradiction may result from different specificities *in vitro* and *in vivo*. Whether the rest of the structure is also involved in the recognition of poly(A) polymerase is more speculative, but it is tempting to suggest that the whole boxed area of Fig. 5 may be involved.

Messenger RNA is known to bind specific cytoplasmic proteins^{20,21} which may be involved in general functions such as the transport of mRNA from the nucleus to the ribosome or control of the degradation of mRNA. The double hairpin-loop structure reported here could also be the recognition site for such proteins but such suggestions must be regarded as speculative in the absence of direct experimental evidence. Sequence studies on other purified mRNAs will reveal how general and therefore how functionally important are these sequence and structural homologies.

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Early Tertiary hiatuses in the north-eastern Indian Ocean

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Cores from drill sites in the Indian Ocean have provided evidence of early Oligocene and early Eocene–Palaeocene hiatuses. That allows a determination of the depth of the carbonate compensation level during the Palaeocene. Possible reasons are given for the occurrence of the early Oligocene hiatus, which may be present at all depths and may well be a world wide phenomenon.

ONE of the major discoveries of the Deep Sea Drilling Project (DSDP) has been the frequent, oceanwide occurrence of discontinuities and hiatuses in the sediment record. Some of these are not just local phenomena but must be related to regional oceanic events^{1–2}.

There are two types of hiatus: first, a hiatus in a continuously cored interval—this is indicated by missing palaeontological zones; second, a hiatus in an uncored or undateable interval. In the second case a hiatus is presumed to exist when the depth–age charts (with age bars showing the time range of each zone) unequivocally indicate that the sedimentation rate over a certain interval (the hiatus) is less than one tenth of the rates in the immediately overlying and underlying sediments.

There is a correlation between depth and age of the oceanic crust which holds in all oceans⁴. Subsidence curves based on this relationship, adjusted for the thickness of sediment between the ooze–clay boundary and volcanic basement^{5,6}, can be used to determine the past depth of the carbonate compensation level, and also the depth range throughout which a hiatus occurred. Some hiatuses in the present sedimentary record are related to patterns of deep water flow^{7,8}. Similarly, the ability to relate some of the older hiatuses to changes in deep and shallow water flow patterns will greatly increase the quantitative understanding of oceanic sedimentation.

Results from Leg 22

The DSDP Leg 22 drill sites are shown in Fig. 1 and lithologies are summarised in Fig. 2. Cores from the Ninetyeast Ridge (Fig. 1, holes 214, 216, 217) showed calcareous oozes overlying a shallow water basal calcareous sequence. Volcanogenic material was also present at sites 214 and 216. The age of the basal sediment increases northwards. Holes 213 and 215, on

either side of Ninetyeast Ridge, show siliceous ooze passing down into brown clay and finally into calcareous ooze.

At drill sites 213, 214, 215, 216 and 217, hiatuses centred on the early Oligocene can be inferred from the recovered cores, and at sites 214, 216 and 217 hiatuses can also be inferred in the early Eocene–Paleocene.

At site 211 two cores taken in a 100-m interval between

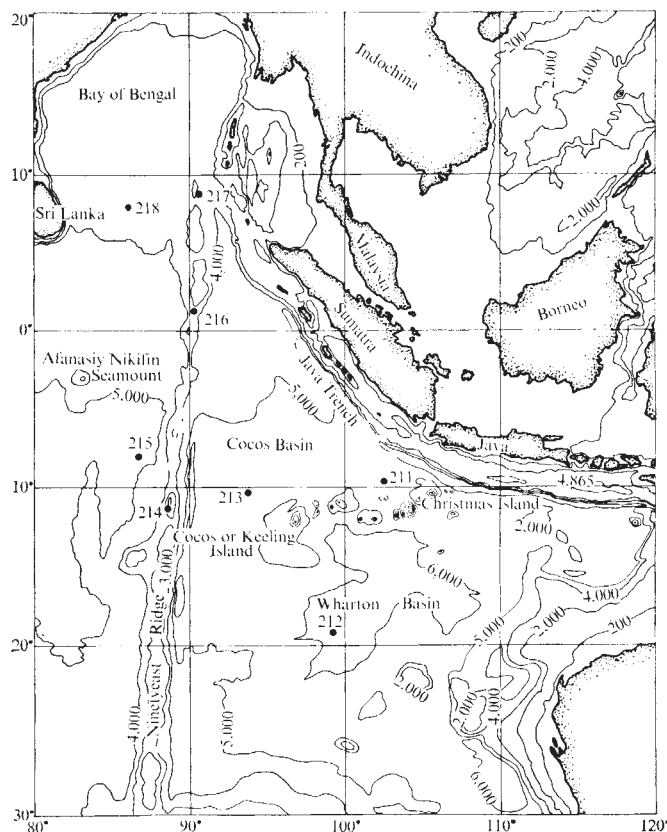


Fig. 1 Leg 22 sites ● plotted on the Russian bathymetric chart of the Indian Ocean (Udintsev, personal communication).

Pliocene and Maastrichtian sediments contained small amounts of barren brown clay. At site 212 there was a total of almost 90 m of brown clay, separated into several units by redeposited calcareous ooze. The clay must have accumulated over a period of about 80–100 Myr. Therefore, at both of these deep water sites hiatuses cannot be inferred from our data (compare with the hiatuses reported in Fig. 1 of ref. 3) because of the poor

fossil control and because the assumed sedimentation rate for brown clay was close to normal over the entire interval in question.

Cores from sites 213 and 215, on either side of Ninetyeast Ridge, imply a major hiatus in the mid Tertiary. At site 213 about 12 m of unfossiliferous clay separates the Middle Eocene from early Middle Miocene—a time span of about 30 Myr;

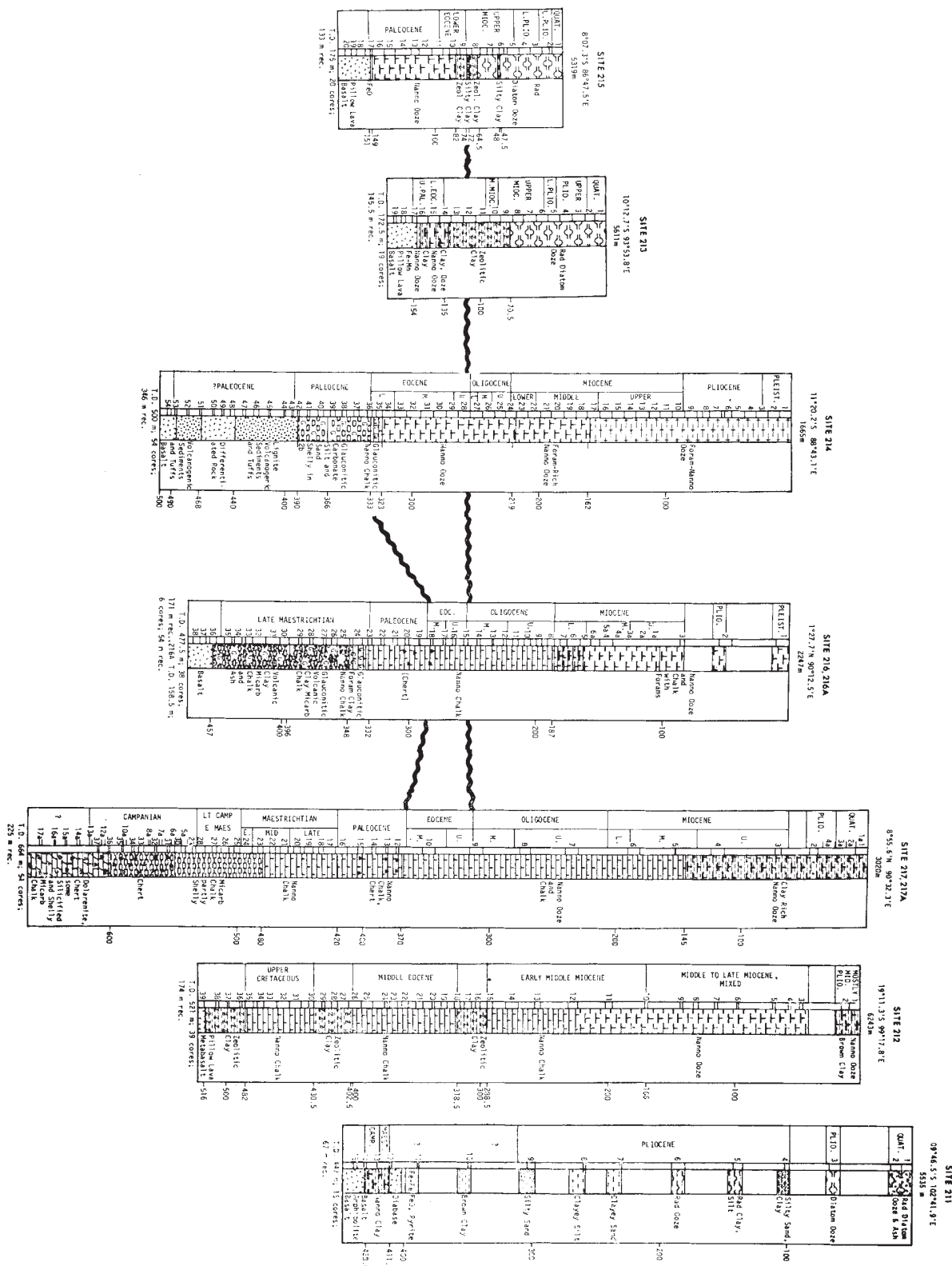


Fig 2 Stratigraphic logs of Leg 22 sites; wavy lines, hiatuses. T.D., total depth.

and at site 215 only 4 m of clay separates early Eocene from late Miocene—a time span of 40 Myr.

At site 214 two cores (20 m) span the entire early Oligocene–late Eocene interval—a period of 10 Myr—so it seems that a small hiatus can be inferred, because the accumulation rate is less than one tenth of the typical rate for pelagic calcareous ooze. At site 216 the nannofossil evidence indicates that the early Oligocene interval is only represented by about 2 m of sediment. One core from site 217 shows a hiatus from the late Eocene to the mid Oligocene.

In the core from site 214, between 336.5 and 334.5 m there is a hiatus of at least 4 Myr between Foraminifera zone P4 (Palaeocene—56 Myr) and zone P7 (early Eocene—52 Myr). At site 216 no early Eocene Foraminifera were recovered, although possible early Eocene nannoplankton were observed in the core catcher sample of one core. The upper portion of that core was Middle Eocene and the core immediately below was Palaeocene. Therefore, at best only a very small part of the early Eocene is preserved. At site 217 the early Eocene and topmost Palaeocene are represented by a very condensed sequence, unless part of the section is missing: Middle Eocene nannoplankton (*Chiasmolithus grandis* Zone) are separated from Palaeocene (*Discoaster multiradiatus* Zone) sediments by a 20-m interval only.

Nature of hiatuses

The major hiatuses in the cores from sites 213 and 215, which are situated in the deep ocean on either side of the Ninetyeast Ridge, are centred close to 35 Myr BP, and the cores from sites 214, 216 and 217, on the Ridge, also revealed smaller hiatuses very close to 35 Myr BP. The cores from the latter sites also indicated small hiatuses between 50–56 Myr BP.

Using the palaeontological dates assigned to the major lithological units (Fig. 2) we plotted the sedimentary record of each site on to oceanic subsidence curves (Fig. 3). For the oceanic sites we computed the expected depth of the site from the empirical curve for normal oceanic crust⁴. We assumed that the sites on Ninetyeast Ridge were formed close to sea level, had a shallow water history, and subsided at the same rate as the Indian plate, to which they were attached¹⁰. We also adjusted the curves for sites 214 and 217 by eye, to give the existing oceanic depth (Fig. 3). From these subsidence curves we have assigned palaeodepths to various features of the sedimentary record.

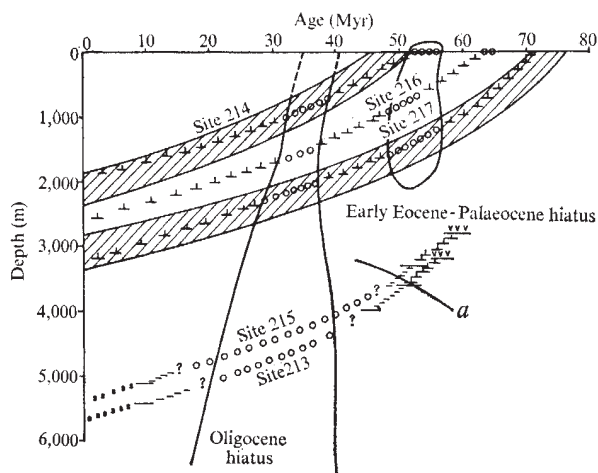


Fig. 3 Geological history of the sites, with hiatuses superimposed on oceanic subsidence curves. a, Carbonate compensation level; ○○○, hiatuses; ||, calcareous sediments; SSS, siliceous ooze; — —, clay; VVV, basalt.

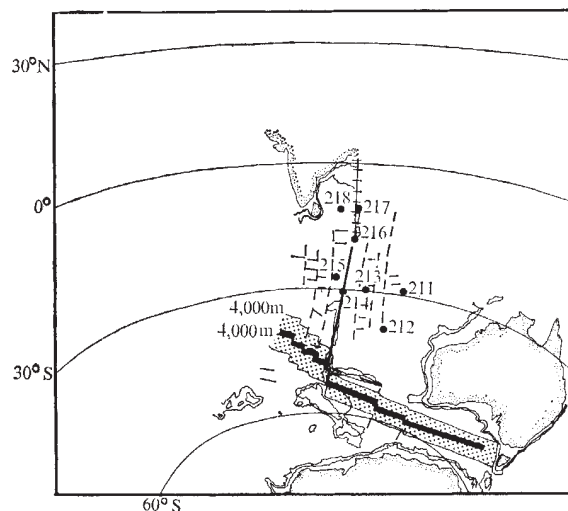


Fig. 4 The region of the ocean floor postulated to be shallower than 4,000 m from the depth-age relationship⁴ superimposed upon the 32 Myr BP reconstruction of the relative positions of Antarctica, Australia, and India¹⁰. Assuming an opening time of 55 Myr BP the depth of the crust between Australia and Antarctica would fall below 4,000 m for the first time approximately 40 Myr BP.

Sites 213 and 215 record a change from carbonate to brown clay deposition at 3,600 and 3,300 m respectively (Fig. 3). This effectively gives the approximate palaeodepth of the Palaeocene level of carbonate compensation in the eastern Indian Ocean.

The major hiatus at sites 213 and 215 occurs at palaeodepths of 4,000–5,000 m. Sites 214, 216 and 217 each show two hiatuses. The younger (Oligocene) occurs throughout depths between 1,000 and 2,500 m, and the older (early Eocene) occurs only at depths shallower than 2,000 m.

It therefore seems that the Oligocene hiatus probably affected, to varying degrees, all of the site positions, irrespective of depth, and also that the Palaeocene–early Eocene hiatus was restricted to depths shallower than 2,500 m.

Evidence from DSDP legs in the Pacific and Atlantic indicates that the Oligocene hiatus is probably worldwide³. We speculate that it may have developed in response to a worldwide readjustment of oceanic circulation. Such a readjustment could be a result of two major events which occurred during the Oligocene: during this Period there was intense glaciation in Antarctica¹¹, and for the first time an effective deep water, circumpolar circulation system could have been established between Australia and Antarctica. Antarctica separated from Australia roughly 55 Myr BP (ref. 12). The subsidence curve data from the oceanic crust just north of Antarctica indicates that this region would have subsided from 2,700 m at the time of separation to more than 4,000 m by 40 Myr BP. Such a depth would allow deep bottom water from further to the west to pass around the north of Antarctica, possibly establishing the Antarctic bottom water circumpolar current (Fig. 4).

We further suggest that the Palaeocene–early Eocene hiatus, which affects only shallow depths on the Ninetyeast Ridge, may be an event related solely to local tectonism.

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letters to nature

Optical variability of PKS0048-097

HOSKINS *et al.*¹ have identified the Parkes radio source 0048-097 with a neutral stellar object of 17 mag, and Carswell *et al.*² have shown that, in common with the BL Lacertae class of quasi-stellar object (BL Lacertids) it has a featureless optical spectrum, that it is a rapid radio variable at high frequencies^{3–6} and that it has a radio spectrum similar to BL Lacertae³. (The name 'Lacertid' has been suggested⁷ for this class, but this invites confusion with meteor showers.) To complete the identification of PKS0048-097, we have investigated two further characteristics of the BL Lacertids: the rapidity of the optical variability; and the total range of optical variability and its correlation with the decimetric spectral index⁸.

Thirty-three blue photographic plates from the Harvard archives, taken between 1921 and 1955 (27 between 1948 and 1955), were measured with a Cuffey Iris Astrophotometer. Relative magnitudes were derived from a reference sequence around the object, which was obtained by a transfer from Mount Wilson Selected Area 117 (see ref. 8). The total amplitude, Δm_{pg} , was found to be 2.7 ± 0.3 mag. The available plates cover only a limited time and so this amplitude should be taken as a lower limit. In addition, on two occasions in 1948 the object exhibited rapid increases in brightness of about 1.4 mag in 55 d and about 0.9 mag in 30 d. Moreover, PKS0048-097 fits an empirical relationship, which exists for BL Lacertids^{8,9}, between Δm_{pg} and the decimetric spectral index α . From the measurements of flux density, S , of Shimmins *et al.*¹⁰ at frequencies, ν , of 408 and 2,650 MHz, the value of α ($= d \log S / d \log \nu$) was found to be 0.33. This value of α should only be considered as representative because of the known radio variability of the source^{3–6}.

It is significant that a value of about 1.5 mag seems to be the lower limit on the total amplitude, Δm_{pg} , for BL Lacertids with a substantial optical record⁹. This empirical result includes PKS0537-441, for which Δm_{yy} is at least 4.9 mag (ref. 11), as well as B20912+29 (ref. 12 and J. T. Pollock, unpublished). We conclude that radio sources with $\Delta m_{pg} \geq 1.5$ mag should be considered as prime candidates for the BL Lacertae class, and that the use of archival photometric records may be an efficient means of identifying new BL Lacertids.

Concerning the nature of PKS0048-097 and the BL Lacertids in general, Oke and Gunn¹³ have shown that the extended object around the quasistellar source BL Lacertae has the spectrophotometric properties of an ordinary giant elliptical galaxy, with a redshift of 0.07. Similar results have been obtained by Wlerick *et al.*¹⁴. Furthermore, if we make the reasonable assumption that the K correction for this galaxy is about 0.1 mag, then it can be shown with the help of the Oke and Gunn data that the BL Lacertae galaxy fits the Hubble diagram of Sandage¹⁵ for radio and N galaxies within the scatter of the points. BL Lacertae is thus associated with an E galaxy that has an intrinsically high luminosity, but is otherwise a normal redshifted object, and it is reasonable to induce that all BL Lacertids have similar properties. The suggestion that these objects are blueshifted¹⁶ is thus unlikely to be

correct, and therefore a major requirement of the local hypothesis for quasars (that they are ejecta from the Milky Way and/or nearby galaxies) apparently cannot be satisfied by the BL Lacertids. It follows that the problem still exists of accounting for the large power radiated by the BL Lacertids and other quasistellar objects at their cosmological distances.

To explain the energy requirements Hjellming¹⁷ has suggested that quasistellar objects are singular 'white holes' which are multiply connected to black holes in our own or some other universe. According to Burbidge¹⁸, the basic concept of white holes originated in the writing of Sir James Jeans in 1929, and has occasionally been revived by Ambartsumian, Hoyle and others. Unfortunately, no theoretical predictions exist for comparisons with observations, and indeed, according to Hjellming (private communication) the hypothesis is highly speculative. Nevertheless, it is well known that most galaxies and many ellipticals have bright semistellar nuclei, and on the basis of morphology it would seem more reasonable to regard the phenomenon of quasistellar objects as an extreme case of bright galactic cores, with which are associated singularities caused by gravitational collapse, rather than white holes.

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Solar models with rotating cores

It is possible to achieve low solar neutrino fluxes with solar models with rapidly rotating cores¹⁻³. But to achieve a flux consistent with the 1 SNU (1 SNU = 10^{-36} neutrino capture per (target atom s⁻¹)) upper limit of Davis⁴, the rotation must be so rapid that the approximate treatment of rotation used is of doubtful validity. Further it is difficult to obtain a result simultaneously giving a flux less than 1 SNU and a solar oblateness consistent with observation⁵. Sakurai⁶ has shown that it is possible for Eddington-Sweet circulation to transfer angular momentum both inwards and outwards in solar models with rotating cores on a time scale much less than the solar lifetime. Thus as the Sun evolves, its rotating core gets smaller while its outer part spins up. This suggestion has led us to investigate the possibility that this spin-up might lead to the onset of some transient phenomenon, such as the sudden beginning of thermal convection. Under special circumstances, a transient state can lead to temporarily depressed neutrino fluxes⁷⁻¹⁰. It was hoped that the transient phase induced by spin-up, coupled with an already depressed flux due to the rotating core would lead to a flux of less than 1 SNU while requiring less extreme transients or rotation than is necessary when these effects are applied separately. In addition, a mechanism for the transient would be provided. Unfortunately this hope has not been realised and additional problems with rotating solar models have been discovered.

As in earlier work, we take rotation into account by multiplying the pressure gradient by a factor containing the average centrifugal force,

$$\frac{dP}{dM} = -\frac{GM(r)}{4\pi r^2} F, \quad F = 1 - \frac{2r^3\Omega^2}{3GM(r)}$$

The radiative gradient, as calculated from the luminosity and used in testing for thermal convection, must be divided by F . According to Sakurai's model, the radiative core of the Sun is initially rotating with a constant angular velocity. Because of induced circulation currents, angular momentum is transported from the surface towards the core. Additionally the outer regions can lose angular momentum to the solar wind. To maximise the final rotation rate, and because the angular momentum transferred to the solar wind depends on the surface rotation rate, we have neglected the surface loss of angular momentum. The Sun as a whole therefore conserves its angular momentum and as the surface layers rotate more slowly, the inner region must rotate more rapidly. The distribution of angular velocity in the transition region between the slowly rotating surface region and the uniformly rotating core region is governed by the condition that it must remain stable against shear flow turbulence. The onset of shear flow turbulence in a stratified medium occurs when the Richardson number

$$Ri = -g \left| \left(\frac{\partial \ln \rho}{\partial r} \right) - \left(\frac{\partial \ln \rho}{\partial r} \right)_{ad} \right| \left(\frac{\partial u}{\partial r} \right)^{-2}$$

is less than some critical value. In this formula g is the acceleration of gravity and $\partial u / \partial r$ is the velocity gradient associated with the differential rotation. We recognise that other instabilities may be associated with the differential rotation, but feel that shear flow turbulence almost certainly will transport angular momentum efficiently even on a short time scale. Thus we have set an upper limit on the velocity gradient by requiring $Ri \geq 1/4$. The width of the rapidly rotating shell is then determined by the condition that this shell contains the angular momentum lost by the outer envelope. Since this shell contains not only its initial angular momentum, but also the angular momentum lost by the envelope, it must rotate more rapidly than the core. We should therefore apply the Richardson condition on both sides of the shell. But in order to maintain a relatively simple computing algorithm and test

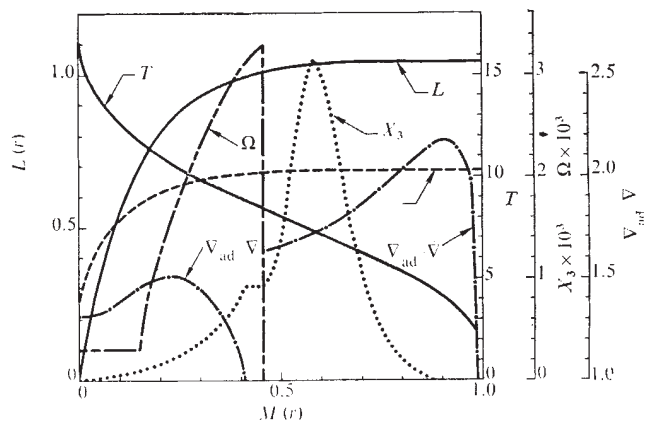


Fig. 1 Structure of sequence-B at an age of 4.7×10^8 yr. Quantities shown are temperature T (10^6 K), hydrogen mass fraction X_3 , rotation rate Ω (rad s^{-1}), ^3He mass fraction X_3 , ratio of adiabatic gradient to actual gradient in the star ∇_{ad}/∇ , and the luminosity L ($L_\odot$). The convective region is the small zone where $\nabla_{ad}/\nabla = 1$. $X = 0.69$; $F = 0.02$; $\Omega_0 = 3 \times 10^{-4}$; $Ri = 0.25$; $\epsilon = 6.4 \times 10^{-3}$; $\Sigma \Gamma \phi = 13.2$.

the importance of satisfying the Richardson condition, we have required the velocity gradient to obey this condition only on the inner side of the shell. On the outer side of the shell we allowed the rotation rate to jump discontinuously to zero. Our procedure then was to choose some uniform initial rotation rate Ω . At later times we assume all the angular momentum exterior to a mass M_Ω has been transferred to the rapidly rotating shell. We assume that M_Ω decreases linearly with time. Our procedure produces a high peak rotation rate and enhances the possibility of inducing a transient. Normally the models were mixed only in those regions where the criterion for thermal convection was satisfied although it would have been more realistic to mix in the region of differential rotation also. The difference between these two cases is small.

The oblateness in each model was calculated following Goldreich and Schubert¹¹. Their equation (14) was integrated from the centre outwards and surface inwards and integrations performed until the oblateness and its derivative were continuous at some matching point. We find oblateness more than an order of magnitude larger than that found by Demarque *et al.*¹ (DMS). We have obtained similar results from programs written independently by each author and have identified the source of the discrepancy. In the models studied there is a discontinuity in Ω at the edge of the rotating core. The equation to be solved contains a term $d\Omega^2/dr$ and thus must satisfy a jump condition at $r_\Omega = r(M_\Omega)$. This is easily accomplished by letting $d\Omega^2/dr = -\Omega_c^2 \delta(r - r_\Omega)$ near the core edge, where Ω_c is the angular velocity at the core edge and $\delta(r - r_\Omega)$ is the Dirac delta function. In our sequence B described below, which is equivalent to DMS sequence C4, we find $\epsilon = 2.8 \times 10^{-3}$ and they find $\epsilon = 1.59 \times 10^{-4}$. If we neglect the jump condition, we find $\epsilon = 1.58 \times 10^{-4}$. Thus it seems that DMS have not properly dealt with the core boundary. We note that the large ϵ would remain if Ω at the core boundary dropped rapidly, but not discontinuously, to zero. As a further check, we made a crude approximation of the model shown in Goldreich and Schubert's Fig. 2 and find an ϵ consistent with theirs.

We have computed two sequences (designated A and B) which are essentially the same as the DMS sequences C2 and C4. The rotating core was handled in the same manner as DMR rather than as described above except that mixing was allowed when the modified criterion for convection was satisfied. Much of the core was convective throughout the sequence. Some of our results for this case are shown in the first two columns of Table 1. The agreement with DMS is remarkable in most respects, even to the agreement of the fluxes from the individual reactions in sequence B. There are, how-

Table 1 Properties of the models

Sequence	A	B	C	D	E
Initial $\Omega/(\text{rad s}^{-1})$	$F = 0.50 = \text{const.}$		3×10^{-4}	3×10^{-4}	3×10^{-4}
Initial X	0.718	0.712	0.69	0.69	0.69
Initial $M_{\Omega}/M_{\odot}$	0.90	0.90	0.98	0.98	0.98
Final $M_{\Omega}/M_{\odot}$	0.07	0.146	0.456	0.456	0.456
Final M uniform rotation/ $M_{\odot}$	0.07	0.146	0.146	0.133	0.073
Ri	—	—	0.25	0.25	0.50
Initial $L/L_{\odot}$	0.19	0.046	0.99	0.99	0.99
Final $L/L_{\odot}$	1.11	1.00	1.05	1.04	1.06
$\log T_c$	—	3.777	3.781	3.782	3.783
$\Sigma\phi/\text{SNU}$	0.50	0.79	13.2	11.3	9.3
X_c	—	0.66	0.248	0.248	0.242
F_{max}	0.50	0.46	0.52	0.52	0.60
$\Omega_{\text{max}}/(\text{rad s}^{-1})$	—	4.5×10^{-3}	3.3×10^{-3}	3.3×10^{-3}	3.0×10^{-3}
Smallest radiative-convective interface	—	0.0	0.422	0.398	0.421
Smallest convective-radiative interface	—	0.161	0.467	0.467	0.450
$\epsilon \times 10^{-3}$	0.46	2.8	6.4	6.4	5.9
Program	RKU	RTR	RTR	RTR	RTR

ever, crucial differences between our results and DMS. It is likely that the angular momentum lost from the core would have been transferred by mass-motion, possibly Eddington-Sweet circulation. Ulrich¹² has pointed out that this would play havoc with the light element abundances at the solar surface. Here we have not even made this assumption and mix only when instability for thermal convection exists. But during the very early stages, the convection zone is deep enough to enhance the surface ^3He from 0 to 1.5×10^{-4} by mass. This is above the upper limit observed by Hall¹³. It will be exceedingly difficult to produce rotating models which do not have such problems with ^3He or the even more sensitive Li and Be. As noted above, we find a much larger oblateness, $\epsilon = 4.6 \times 10^{-4}$ and 2.8×10^{-3} compared to their $\epsilon = 2 \times 10^{-5}$ and 1.6×10^{-4} . Our results are in strong disagreement with the observed value⁵ of $\epsilon = 5 \times 10^{-5}$, which can be taken at least as an upper limit.

There are several possibilities for inducing a transient phase with a core that is spinning up. First, we suppose that the outer part of the rotating core arrives at some part of the ^3He peak after 4.7×10^9 yr of evolution. This could well cause the simultaneous onset of thermal convection. Because of the stored energy in the ^3He and the modified radiative gradient inside the rotating core, it is conceivable that the convective region might grow. But our calculations show that mixing

never occurs except for rotation rates almost as large as those used by DMS.

The results for one model (designated C) which developed a convective region are shown in the second column of Table 1. The initial rotation rate was $3 \times 10^{-4} \text{ s}^{-1}$. While evolving for 4.7×10^9 yr the rotating core was reduced from $0.98 M_{\odot}$ to $0.46 M_{\odot}$. At this point, a small convective region developed. Figure 1 shows the structure at this time. Comparison of our curve for Ω and Sakurai's Fig. 2 emphasises the extremes we must have to get any effect. The convective region is on the inner slope of the ^3He peak. Further evolution with small time steps and no further reduction in the rotating core size show that the convective region does not grow, but rather shrinks in size. The curve for the ratio of the adiabatic gradient to actual gradient in the star, $\Delta_{\text{ad}}/\Delta$ in Fig. 1, shows that the central core is extremely stable.

There are further problems with this model. To achieve the proper luminosity, X had to be reduced to 0.69 initially. Thus the neutrino flux ends up at 13.2 SNU which is more than double our standard Sun. Further, the oblateness of this model is more than 100 times larger than Dicke's value. Certainly any model which has any substantial rotational modification in the ^3He peak will suffer similar oblateness problems.

The results for a similar model in which the composition was mixed in the differentially rotating region is shown in column 4 of Table 1. It differs little from the previous model.

One additional possibility remains: if the differentially rotating region reaches into the central region of high nuclear burning, the addition of ^3He from the outer part of the model might lead to further mixing driven by nuclear burning. Such a sequence (designated E) is shown in column 5 of Table 1. Mixing has been allowed in the differentially rotating region and the inner edge of this region is at $0.073 M_{\odot}$. The mixed region has been extended over a larger mass than sequence D by making the Richardson number larger. The region unstable to thermal convection extends from 0.421 to $0.450 M_{\odot}$ and again has no inclination to grow. The oblateness is still huge.

So it seems that the only possibility for the spin-up to induce a neutrino-eliminating transient would be for the rate of spin-up to be extremely fast. M_{Ω} would have to be reduced from say $0.6 M_{\odot}$ to $0.1 M_{\odot}$ in a time comparable to the ^3He lifetime at the centre ($\sim 10^6$ yr). This rate of spin-up seems implausibly high.

The models proposed by DMS seem to be ruled out as solutions to the solar neutrino problem. (Roxburgh does not describe his models in sufficient detail for us to check, although they seem very similar to those reported by DSM.) Their oblateness is much greater than the observed value. Their ^3He at the surface is above the observed upper limit. Finally a problem which will afflict all such models is demonstrated

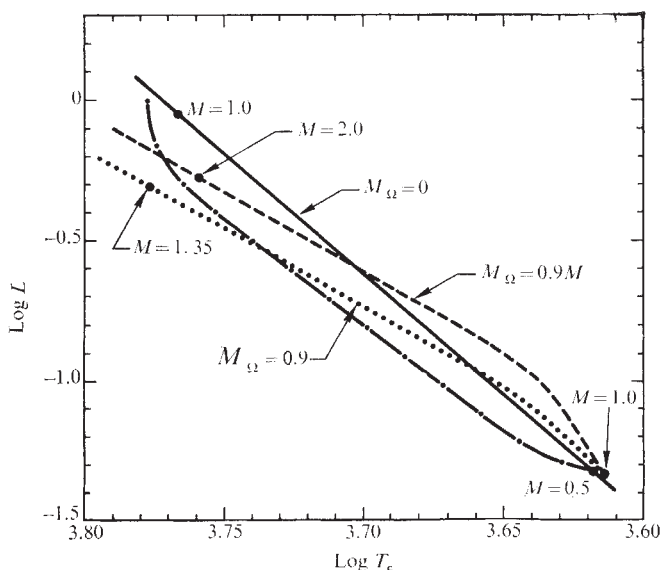


Fig. 2 H-R Diagram for main sequence models for the composition $X = 0.712$. The solid line is the non-rotating main sequence, the long dashed line has $M_{\Omega} = 0.9 M_{\odot}$, the short dashed line has $M_{\Omega} = 0.9 M$; in the rotating cores $F = 0.50$. Reference marks ($M_{\odot}$) are shown on each sequence. The evolutionary track for model B is also shown (dot-dash line).

in Fig. 2. A non-rotating main sequence is shown along with ones with two different values of $M\Omega$ and the evolutionary tracks for sequence B. In a cluster of stars similar to the Sun one might expect to find stars scattered throughout the region between the non-rotating main sequence and one of the rotating ones. A typical spread at a given T_e is 0.2 to 0.3 in $\log L$. This spread of 0.75 mag is not observed in Praesape¹⁴, for instance.

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Oblateness of solar models with rotating cores

ROOD and Ulrich¹ have discussed the oblateness of solar models with a rotating core. They find a larger oblateness for the same model than we did². We have looked again into the oblateness calculation and now believe that the correct solution of the equations of Goldreich and Schubert³ gives an oblateness for the final model of our² sequence C4 in agreement with the value derived by Rood and Ulrich. We also agree with Rood and Ulrich's explanation for the discrepancy.

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Geomagnetism and the tropospheric circulation

IN a recent article in *Nature*¹, King draws attention to certain similarities between the atmospheric flow pattern in mid-troposphere and the Earth's magnetic field. He postulates "some unknown magnetic-field dependent mechanism" which exerts a control on the tropospheric circulation and associated atmospheric pressure field. The evidence on which he bases this idea consists of the similarity between the distribution of the height of the 500-mbar pressure surface in the atmosphere and that of the geomagnetic field strength, particularly in latitude 60°. He supports this with some indication that the features of the 500-mbar surface have moved westwards in parallel with the features of the geomagnetic field.

The purpose of this communication is to point out that it is unnecessary, and probably misleading, to postulate any causal relationship between the geomagnetic field and the 500-mbar contours, because the main features of the maps of 500-mbar contours can be explained by direct calculation from physical and dynamical principles without consideration of magnetic effects. Moreover any westward movements of the features of the

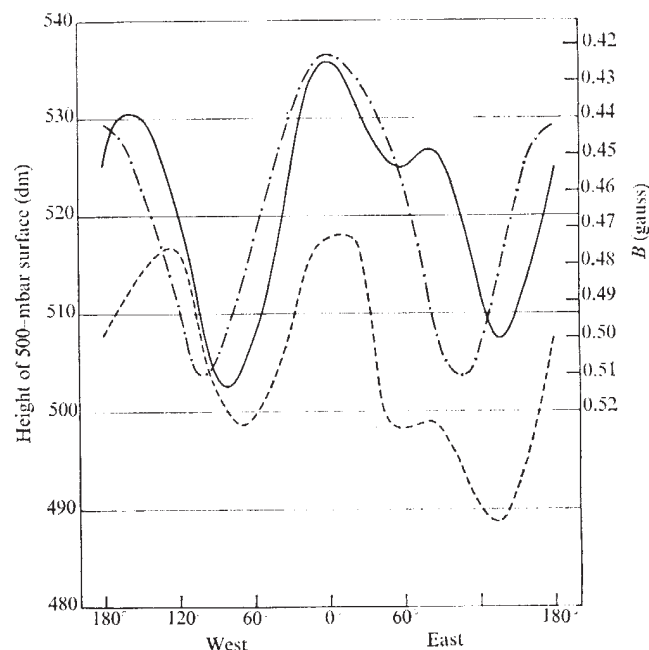


Fig. 1 Longitudinal variation of 500-mbar height and magnetic field strength along latitude 60°N in January. — Observed 500-mbar height from ref. 6; --- dynamically computed 500-mbar height; - - - magnetic field strength from Fig. 2 of ref. 1.

500-mbar height field appear to be merely the result of temporary fluctuations, and therefore unlikely to be linked with the westward motion of geomagnetic features which has continued over a century or more.

In recent years a great deal of effort has been devoted to the numerical solution of the equations of motion of the atmosphere, together with the thermodynamic equations, equations for radiative transfer and the mathematical representation of the other physical processes in the atmosphere. Given the external heat sources—the solar radiation—and appropriate thermal and mechanical boundary conditions at the Earth's surface, the aim has been to calculate the global atmospheric circulation and such related features as the distribution of temperature and pressure. The atmospheric circulation is far from being steady,

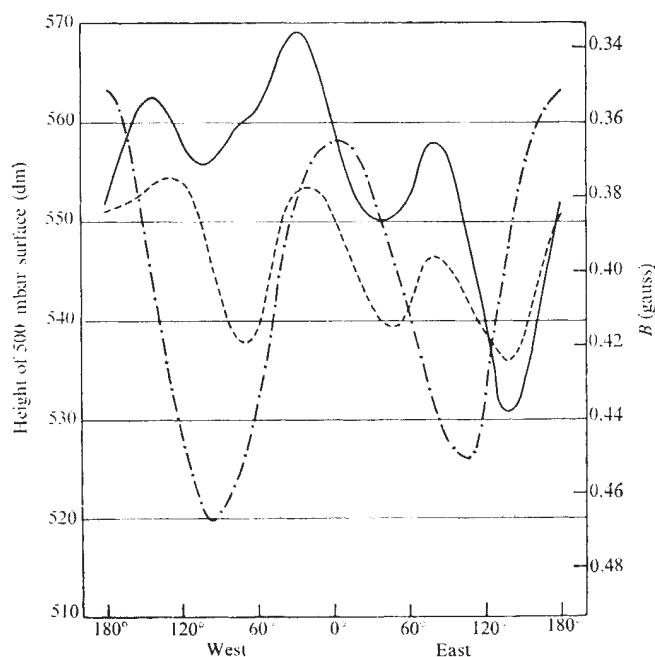


Fig. 2 Longitudinal variation of 500-mbar height and magnetic field strength along latitude 40°N in January. (Explanation as Fig. 1.)

and the short period disturbances—the depressions and anti-cyclones—play an essential role in maintaining the average circulation. The numerical calculations reproduce this feature. Starting from an arbitrary initial state, and integrating with respect to time, they reproduce the irregularly fluctuating character of the atmosphere. The mean seasonal circulation can be obtained by averaging over a period of some 30 d or more. A considerable degree of success has been obtained in such calcu-

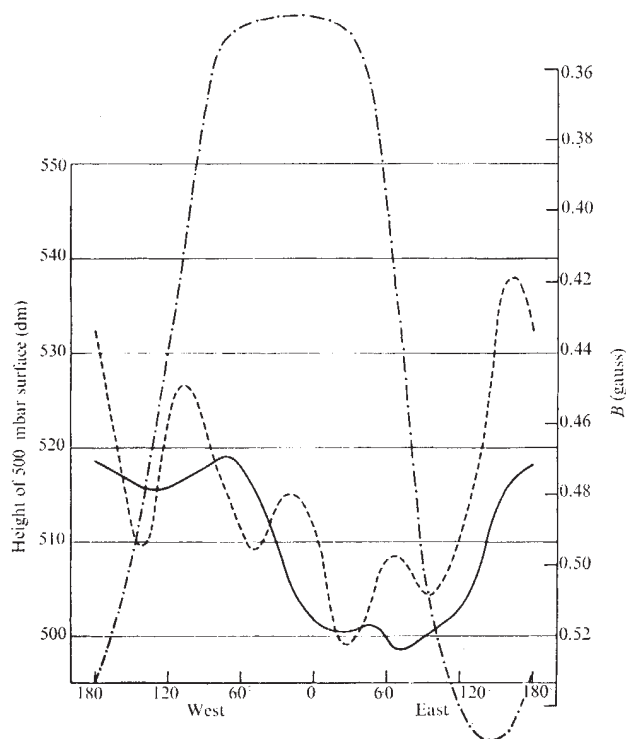


Fig. 3 Longitudinal variation of 500-mbar height and magnetic field strength along latitude 60°S in July. (Explanation as Fig. 1 except that observed 500-mbar height is taken from ref. 9.)

lations and full reports have been published by several independent research groups²⁻⁴.

To illustrate this, I have taken the calculated profile of 500-mbar height around latitude 60°N from an integration performed during the course of one such numerical experiment with the model of Gilchrist, Corby and Newson⁵ and plotted it in Fig. 1 along with the observed January profile as mapped by Heastie and Stephenson⁶. All the main features are fairly well reproduced although there are some small mislocations and incorrect intensities of the troughs and ridges. (The geomagnetic field strength B at 400 km is reproduced from ref. 1 for comparison.) It is noteworthy that the dynamical calculations reproduce the weak ridge at 80°E whereas there is no corresponding feature in the geomagnetic field. It is also noteworthy that the 500-mbar profile around latitude 40°N is also reproduced fairly well by the calculations (Fig. 2) although here

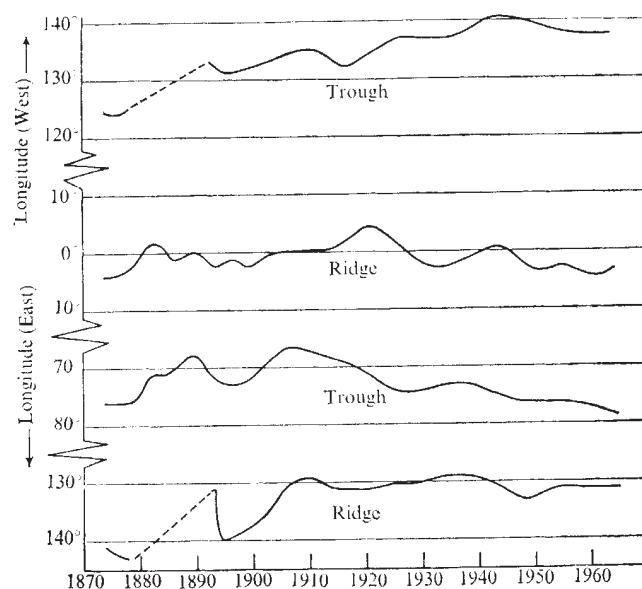


Fig. 4 Longitude of the main 500-mbar troughs and ridges at 500-mbar as a function of time based on 10-yr running means (December–February).

the dominant wave number is three and not two as in the geomagnetic field.

Figure 3 shows similar profiles for latitude 60°S, also in winter. Here the observations are probably inadequate to define the observed field with any reliability but the agreement between calculated and observed distribution is such as to suggest that a geomagnetic effect need not be invoked to explain the main features of the profile and in particular the dominant wave number one.

King¹ uses a comparison of two sets of climatological charts as evidence for the westward movement of the features of the 500-mbar height field between the 1930s and 1950. The data available for the preparation of the earlier charts does not really warrant such a comparison, indeed the second set were prepared as a replacement of the earlier set when more data became available, and I am sure that the authors of the earlier charts would attribute the differences to lack of data rather than to any real change. More recently, in the course of research in long-range forecasting, a set of monthly charts of the 500-mbar heights for each year since 1874 has been built up in the Meteorological Office. In the earlier periods, when data for the

500-mbar level were lacking, these charts are obtained by a correlation procedure based on the surface pressure chart for which adequate observations are available. Figure 4 shows the changes in the longitude of the main 500-mbar troughs and ridges as a function of time. This diagram is based on 10-yr running means for the winter period (December–February). Although there is some confirmation of a westward displacement of these features (except the East Asiatic trough) in the period leading up to 1950, the pattern is one of irregular movements during the whole period, without much overall displacement. On the other hand the movement of the geomagnetic features has been believed to be westwards (albeit irregularly) at least since the early nineteenth century^{7,8}. It does not, therefore, seem reasonable to seek an association between the erratic movements of these atmospheric features and the more or less steady progression of the geomagnetic features.

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DR KING REPLIES—It is widely believed that long term changes in the circulation of the lower atmosphere will be accompanied by climatic variations. The causes of these changes have not yet been established and at the present time, therefore, they are not predictable. I and other workers (see ref. 1) have suggested that the Earth's magnetic field may influence, through some unknown mechanism, the behaviour of the lower atmosphere at high latitudes in winter. Examples showing the type of evidence on which this suggestion is based are shown in Fig. 1; further evidence will be published elsewhere. I pointed out that such magnetometeorological evidence "cannot be construed as proof that the Earth's magnetic field and the average tropospheric pressure patterns are related, but it does suggest that more work on this problem should be undertaken."

Sawyer² claims that "it is unnecessary, and probably misleading, to postulate any causal relationship between the geomagnetic field and the 500-mbar contours . . ." Several major objections can be made to this claim:

(1) It is based on the belief that the Meteorological Office model yields theoretical results in such good agreement with observations that it is unnecessary to postulate the existence of hitherto unconsidered relationships which might exist between the lower atmosphere and, for example, the geomagnetic field. Not all atmospheric modellers, however, would accept that current models yield such satisfactory results. Smagorinsky³, for example, says in the introduction to a GARP volume describing the 19 meteorological models currently known to exist: "All present models are sufficiently different from the real atmosphere so that, when real data are used with any one of them . . . the conclusions are highly model-dependent." He continues: "The traumatic response of a model to alien data, for example conditioned by another model or, even worse, by the atmosphere itself, may often take the form of outright rejection of the

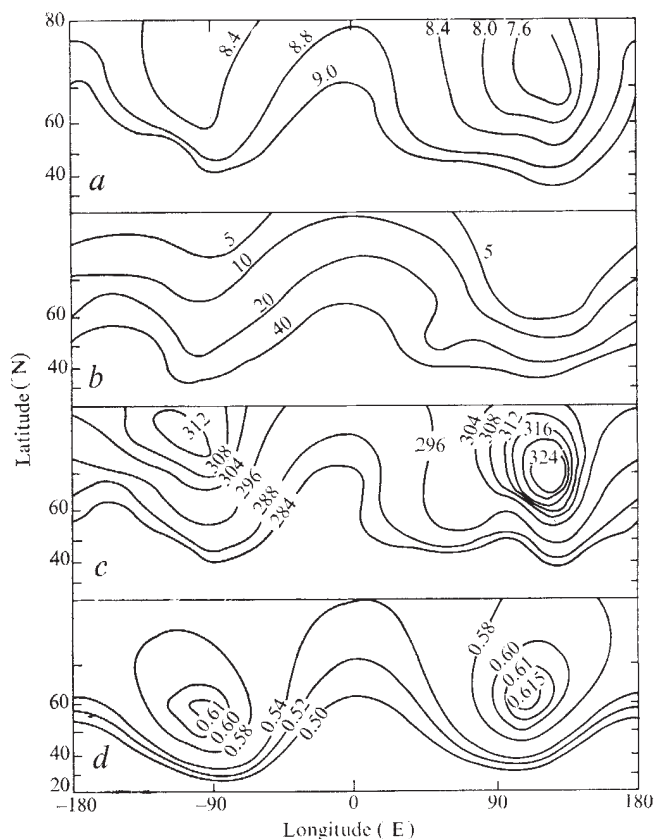


Fig. 1 a, b, c, Northern Hemisphere average radio refractivity maps, compiled by Bean *et al.*¹⁰ from radiosonde data for November 1958–62. a, Radio refractivity 'dry term' tropospheric scale height (km); b, mean sealevel radio refractivity, 'wet term'; c, mean sealevel radio refractivity, 'dry term'. The maps effectively illustrate the spatial variations of, respectively, the average tropospheric temperature, sealevel humidity and sealevel air density. d, Variation of total magnetic intensity (c.g.s. units) at the Earth's surface¹¹.

transplant attempt." These statements indicate that even the best of the available models do not produce results in satisfactory agreement with the real atmosphere. Manabe and Terpstra⁴, after a major study carried out using the general circulation model developed at the NOAA Geophysical Fluid Dynamics Laboratory, concluded that as far as the Northern Hemisphere is concerned: "The agreement between the distributions of geopotential height of the 500-mbar level surface in the mountain-model atmosphere and that in the actual atmosphere is particularly poor in higher latitudes." These are the latitudes for which I suggested that the geomagnetic field may exert a controlling influence on the tropospheric circulation¹. As far as the Southern Hemisphere is concerned, Manabe and Terpstra⁴ reported: "The simulation of the flow field has much to be desired" and "The agreement between the computed and observed (pressure maps) is poor". The results of the calculations were judged to be "not very realistic" because "the intense surface westerlies in the middle latitudes of the Southern Hemisphere are almost missing" in the results obtained using the mountain model.

Some measure of the disagreement can be obtained by noting that the correlation coefficient between the observed and calculated 500-mbar height values at 60°N in January (from Fig. 1 of ref. 2) is only 0.69. The correlation coefficient between the observed 500-mbar data (shifted 25° westwards in order to allow for the height-dependent phase shift of the pressure variations) and the total intensity of the Earth's magnetic field at the Earth's surface is, however, –0.90, while that between

the January 500-mbar heights (phase-shifted) given by Palmen and Newton⁵ and the intensity of the magnetic field for 60°N is -0.963. Such high correlation coefficients are very unusual and, although they do not prove anything, their implications should not be dismissed lightly. The modelling work referred to above clearly suggests that the present understanding of the atmosphere embodied in the models is inadequate and that further interdisciplinary research is needed to discover what has been omitted from the models.

(2) Sawyer suggests that the numerical work which he refers to is based only on physical and dynamical principles and that it does not, therefore, take into account magnetic effects. This is not necessarily true; the results were obtained using the model of Gilchrist *et al.*⁶ which incorporates, as a necessary boundary condition, values of the observed sea surface temperature which were held constant throughout the calculations. The temperature map used by Gilchrist *et al.* is such that the 6° C isotherm moves 21° towards the north (from 45°N to 66°N) while crossing the Atlantic from America to Europe and 18° northwards while crossing the Pacific from Asia to America. It is obviously not possible to use the results of calculations which incorporate such boundary conditions to decide whether the Earth's magnetic field or any other external phenomenon influences the circulation of the atmosphere.

(3) Sawyer claims that the main features of the 500-mbar maps are explained by numerical modelling; one feature of the maps which numerical work clearly cannot yet explain, however, is that they change on a climatic time scale. Present thinking on the usefulness of numerical modelling in climatic studies is contained in ref. 7. This report, prepared for a session of the Joint Organising Committee for GARP, says: "It is evident that detailed mathematical simulation of the atmosphere's three-dimensional behaviour cannot be the sole avenue to the study of long period variations of climate (for example, a period of the order of a decade or longer) and that the structure of the current general circulation models is unsuitable for that purpose."

McIntosh and Thom⁸ have summarised the current usefulness of numerical studies for climatic purposes as follows: "The synoptic and analogue methods of 30-day forecasting do not appear capable of being developed further to any significant extent. It is scarcely an exaggeration to say that in all other respects the meteorologist has, at present, nothing positive to contribute concerning future long range weather prospects. This situation is very unlikely to change until numerical studies of the general circulation are much further advanced than now."

These two quotations draw attention to the need for more knowledge about the processes which will alter the circulation of the atmosphere. One way of finding whether the magnetic field and the weather are related is to undertake, as I suggested¹, "further comparisons of meteorological data from widely spaced epochs for which reliable maps of magnetic field strength are also available". Such work will require reasonably accurate magnetic information because the movement of features of the magnetic field pattern are by no means uniform. The manner in which the pattern changes is in fact extremely complex⁹ and Sawyer's representation of the motions of the geomagnetic anomalies as a "more or less steady progression of geomagnetic features" which are unlikely to be associated with the observed irregular secular movements of atmospheric features is unjustified.

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Initiation of the proto circum-Antarctic current

THE planktonic foraminiferal species *Guembelitra stavensis* Bandy (Fig. 1) lived in the austral gulf sea between Australia and Antarctica during the Oligocene, and with the final separation of the two continents at the eastern hinge of the South Tasman Rise, it spread out eastwards into the south-western Pacific. The palaeogeographical distribution and the very short stratigraphic range of *G. stavensis* in the south-western Pacific provides the first palaeontological evidence of the initiation of the proto circum-Antarctic current in the lower part of the Upper Oligocene.

It has been suggested that the circum-Antarctic current first became operative during either the late Oligocene¹ about 30 Myr BP, the Middle Oligocene^{2,3}, or the Lower Oligocene⁴. The first two suggestions were based apparently on interpretations of a late Palaeogene regional unconformity in the Tasman and Coral seas and the third was based on an interpretation of Oligocene planktonic Foraminifera from New Zealand.

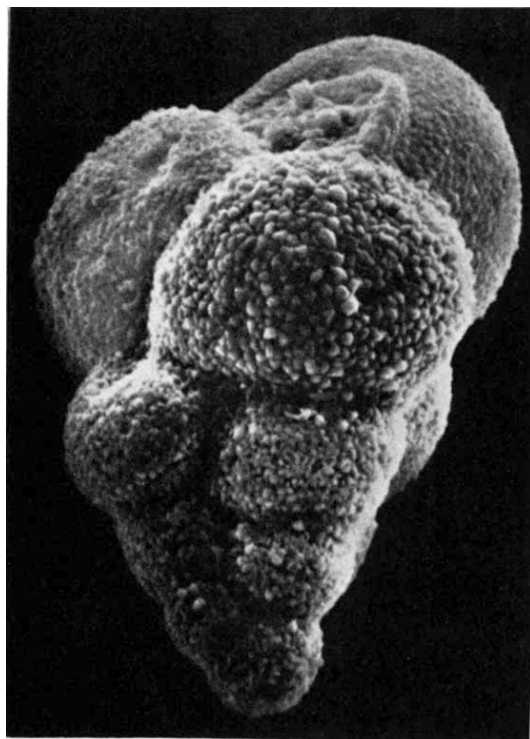


Fig. 1 *Guembelitra stavensis* Bandy, $\times 560$; site 276 DSDP Leg 29 (latitude 50° 48.11'S; longitude 176° 48.40'E).

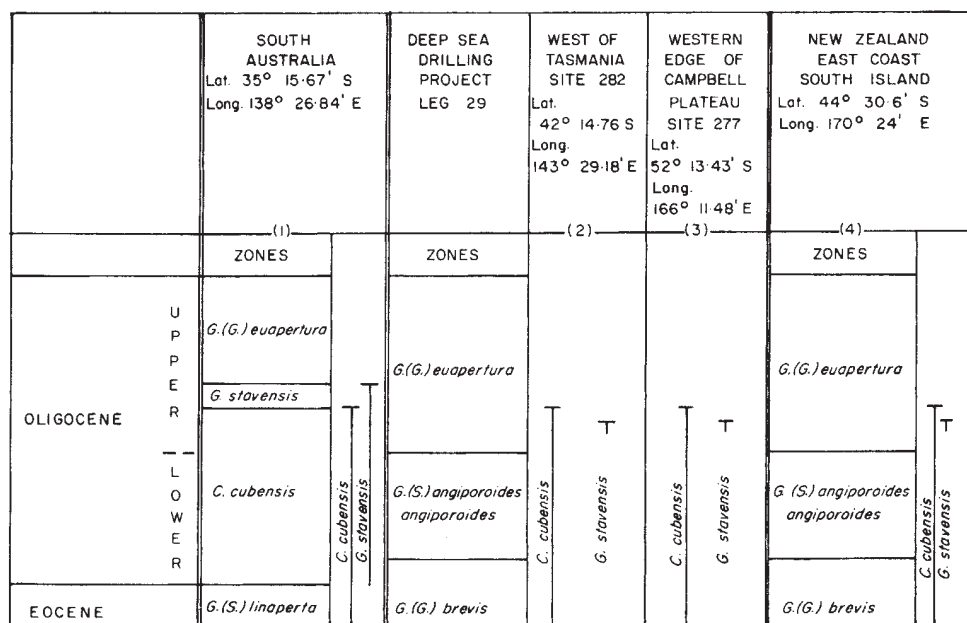


Fig. 2 Stratigraphic records of *G. stavensis* in South Australia, two sites of DSDP Leg 29 and one from New Zealand.

The palaeontological dating of the unconformity is basic to the original hypothesis. According to Kennett *et al.* "The unconformity is centred approximately in the *Globigerina angiporoides* and *Globigerina brevis* foraminiferal zones⁹ but frequently includes the *Globigerina linaperta* to *Globigerina euapertura* zones⁹; or the *Isthmolithus recurvus* to *Reticulofenestra placomorpha* zones¹⁰ (late Eocene to Middle Oligocene)." But calcareous nannofossil *I. recurvus*–*R. placomorpha* zones have been correlated with the *G. (S.) linaperta* to *G. (S.) angiporoides angiporoides* zones in New Zealand⁵, that is the nannofossil zones are older than the *G. (G.) euapertura* zone and the erosion which formed the unconformity must also be older than that zone.

The stratigraphic and palaeogeographic distribution of *Guembelitra stavensis* which was originally described from the Middle Eocene of Alabama seems to fix the stratigraphic position of the initiation of the proto circum-Antarctic current.

Guembelitra stavensis lived in the southern parts of South Australia during Lower to Upper Oligocene *Chiloguembelina cubensis* – *G. stavensis* zones times^{6,7} and became extinct in the equivalent of the lowermost *G. (G.) euapertura* zone (Fig. 2). It lived slightly longer there than in areas to the east, however. Before it became extinct, *G. stavensis* migrated eastwards and is recorded at this stratigraphic level west of Tasmania at the Deep Sea Drilling Project (DSDP) Leg 29, site 282 (Figs 2 and 3).

With the establishment of open sea conditions following the separation of Tasmania and its southern continental extension from Antarctica, *G. stavensis* apparently spread rapidly eastwards. It is recorded for only a short interval in the lowest part of the *G. (G.) euapertura* zone on the western edge of the Campbell Plateau at DSDP site 277 (Figs 2 and 3). *Guembelitra stavensis* has also been encountered at DSDP site 276 on the eastern edge of the Campbell Plateau (latitude 50° 48.11'S; longitude 176° 48.40'E) and was obtained from a bit-sample presumed to be from the same stratigraphic level in the *G. (G.) euapertura* zone. *G. stavensis* has also been recorded at the same stratigraphic level on the eastern coast of South Island, New Zealand (Figs 2 and 3) but it has not been recorded either north of this locality in the New Zealand Oligocene, or in the Tasman Sea sediments. A southward migration is therefore discounted.

I postulate that the migration of *G. stavensis* eastwards from the region of South Australia into the South Tasman Sea and south-western Pacific was permitted by the initiation of the proto circum-Antarctic current. The relatively short stratigraphic range of *G. stavensis* at sites east of South Australia—appeared and became extinct just before the extinction of *C. cubensis* Palmer in the lowest *G. (G.) euapertura* zone—can be placed on the radiometric scale. Berggren⁸ has recorded the extinction of *C. cubensis*, termed the 'Chiloguembelina extinction Datum', in about the middle of the Oligocene *Globigerina angulatus* zone. Records of K/Ar dates on the type Chattian⁸ of the North German Basin give a range of 28.8–31.2 ± 1.5 Myr BP, and Berggren⁸ has placed the extinction of *C. cubensis* at 28 Myr. Supporting evidence for this date has come from an examination of basalts in Victoria, Australia⁹, similarly dated at 26.5–27 Myr BP, which were interpreted as having been emplaced somewhat later than the locally recorded extinction of *C. cubensis*.

Thus, accepting the validity of the radiometric dates and the correlation of the extinction level of *C. cubensis*, the initiation

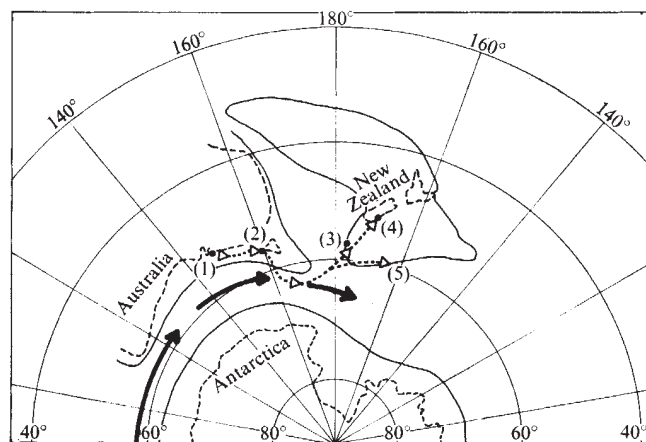


Fig. 3 Tentative migration route of *G. stavensis* (heavily dashed line) after the initiation of the proto circum-Antarctic current during the Upper Oligocene lowest *G. (G.) euapertura* zone. The shapes of continental areas (solid lines) are based approximately on the present 4,000 m bathymetric contour¹⁰ (dashed lines show present-day continental boundaries). Details of present-day localities (1)–(4) are given in Fig. 2. Locality (5) is at latitude 50° 48.11'S; longitude 176° 48.40'E, DSDP Leg 29, site 276. Heavy arrows, proto circum-Antarctic current.

of the proto circum-Antarctic current probably occurred between 27 and 28 Myr BP and that event was followed by the eastward migration of *G. stansis*.

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Advance of the Greenland Ice Sheet on to north-eastern Ellesmere Island

GEOLOGICAL evidence suggests that an important restricted ice advance occurred along north-western Greenland and north-eastern Ellesmere Island during the last glaciation.

Information on past ice margins is critical to the understanding of ice cores, delimitation of refugia, and preservation of both glacial and marine features that span several glaciations. It is widely assumed that during the last glaciation the Greenland Ice Sheet reached north-eastern Ellesmere Island^{1,2}. It has also been assumed that during the last glaciation the ice sheets over northern Ellesmere Island and north-western Greenland coalesced to form a ridge 700–800 km wide centred over Kane Basin with outlet ice draining to the north-east and south-west along the line of Nares Strait³. As yet, however, little geological evidence has been collected along these ice margins in relation to ice cores, and important existing literature has been ignored^{4,5}.

North-eastern Ellesmere Island and north-western Greenland are separated by a distance varying from only 40 km to 130 km (Fig. 1). On north-eastern Ellesmere Island a major centre of ice dispersal exists over the United States Range where piedmont glaciers descend southwards along the northern edge of the Hazen Plateau. The southern margin of the active, United States Range Icefield is only 150 km to the north-west of glaciers from the Greenland Ice Sheet. The Hazen Plateau extends another 75 km to the south-east where it terminates directly across from Polaris Promontory, north-western Greenland. Physiographically, therefore, the region around the Hazen Plateau and the Polaris Promontory represents a relatively open area for the former interaction of the Ellesmere Island and Greenland ice sheets (Fig. 1).

Taylor⁶ proposed that northern Ellesmere Island was once covered by the Greenland Ice Sheet, but this was rejected by Smith⁷ on the basis of the distribution of Ellesmere erratics near the summits of the United States Range (Fig. 1). Christie⁸, however, noted the presence of red granite and gneiss erratics on Judge Daly Promontory and along the eastern edge of the Hazen Plateau, and suggested that a minor extension of the Greenland Ice Sheet on to north-eastern Ellesmere Island occurred at some unknown time in the past (Quaternary or Upper Tertiary glaciations?).

Red granites are exposed in Inglefield Land, north-western Greenland, and also amongst the moraines of Polaris Promontory⁸ and the glacial deposits south of Independence Fiord, in north-eastern Greenland⁹. The hypothesis that the red granites were deposited by the Greenland Ice Sheet, which was possibly confluent with the south-eastward moving Ellesmere Island Ice Sheet over the eastern edge of the Hazen Plateau, implies that the granite erratics fall within a contained boundary. No detailed information yet exists, however, on the regional presence or absence of granite erratics over larger areas of northern Ellesmere Island.

Granite and gneiss bedrock has also been observed on the extreme northern coast of Ellesmere Island, and additional outcrops may extend inland beneath the United States Range Icefield. These would then coincide with the maximum, measured Pleistocene ice thicknesses over northern Ellesmere Island; and the southward movement of ice⁷ may thus have deposited these erratics on the outer Hazen Plateau and Judge Daly Promontory. This alternative hypothesis of a northern Ellesmere Island provenance is speculative and does not preclude the possibility that the Greenland Ice Sheet could have deposited the erratics⁸. Assuming that the red granite erratics on Ellesmere Island are products of the Greenland Ice Sheet then the remaining question concerns the timing of this ice advance.

Along the southern margin of the Hazen Plateau a discontinuous system of moraines marks the terminal position of ice draining out of the United States Range during the last glaciation¹⁰ (Fig. 1). This system, termed the Hazen Moraines¹⁰, is associated with a maximum date of $8,130 \pm 200$ BP and is correlative with the Cockburn Stade of eastern Baffin Island¹¹. Another till, of unknown age, extends above and beyond the Hazen Moraines and was deposited by an advance of the north-eastern Ellesmere Island ice out to Robeson Channel (Fig. 1). Shells adjacent to a coastal moraine, which is correlated with this till, date at $27,950 \pm 5,400$ BP. This date, and the advanced surface weathering on a nearby ice-contact terrace, suggest that the advance may predate the last glaciation on northern Ellesmere Island. This most extensive northern Ellesmere Island ice advance is referred to as the Defosse Glaciation¹⁰. The preservation and lack of overriding of its coastal moraine and ice-contact terraces, suggest that any advance of the Greenland Ice Sheet on to north-eastern Ellesmere Island would have to predate the Defosse Glaciation.

In Inglefield Land (Fig. 1) there are three distinct glacial advances⁴. The oldest till (Stage 1) is the most extensive and reaches out to Kennedy Channel. It is characterised by iron-stained, silicate rocks, and limestone boulders with solution cavities. The Stage 1 till is possibly correlative with the advance of the Greenland Ice Sheet on to north-eastern Ellesmere Island^{4,8}. The Stage 2 till, less extensive than the Stage 1 till, is oxidised to a depth of one metre. Stage 3 defines the least extensive and most recent ice advance on Inglefield Land and is marked by a semicontinuous moraine system. A large terrace, possibly contemporaneous with, or older than, the Stage 3 ice advance, contained an organic layer dated at $20,800 \pm 2,900$ BP. The distribution and relative ages of the three glacial stages on Inglefield land, therefore, indicate that a minor advance took place in this locality during the last glaciation and that the advance of the Greenland Ice Sheet on to north-eastern Ellesmere Island would have to be as old as Tedrow's⁴ Stage 1 till.

Davies⁵ also notes at least three glaciations on northern Greenland during the Pleistocene⁵. The oldest glaciations are believed to be pre-Wisconsin in age and successively less extensive toward the present. Two stages of glaciation occurred on Polaris Promontory and again the oldest is the most extensive and is considered to pre-date the latest Würm-Wisconsin glaciation⁵. Karlstrom also suggests (personal communication) that low levels of glacial intensity occurred during the last major glaciation over Polaris Promontory. He adds that the Greenland ice may have approached, but not merged with, the Canadian ice during the

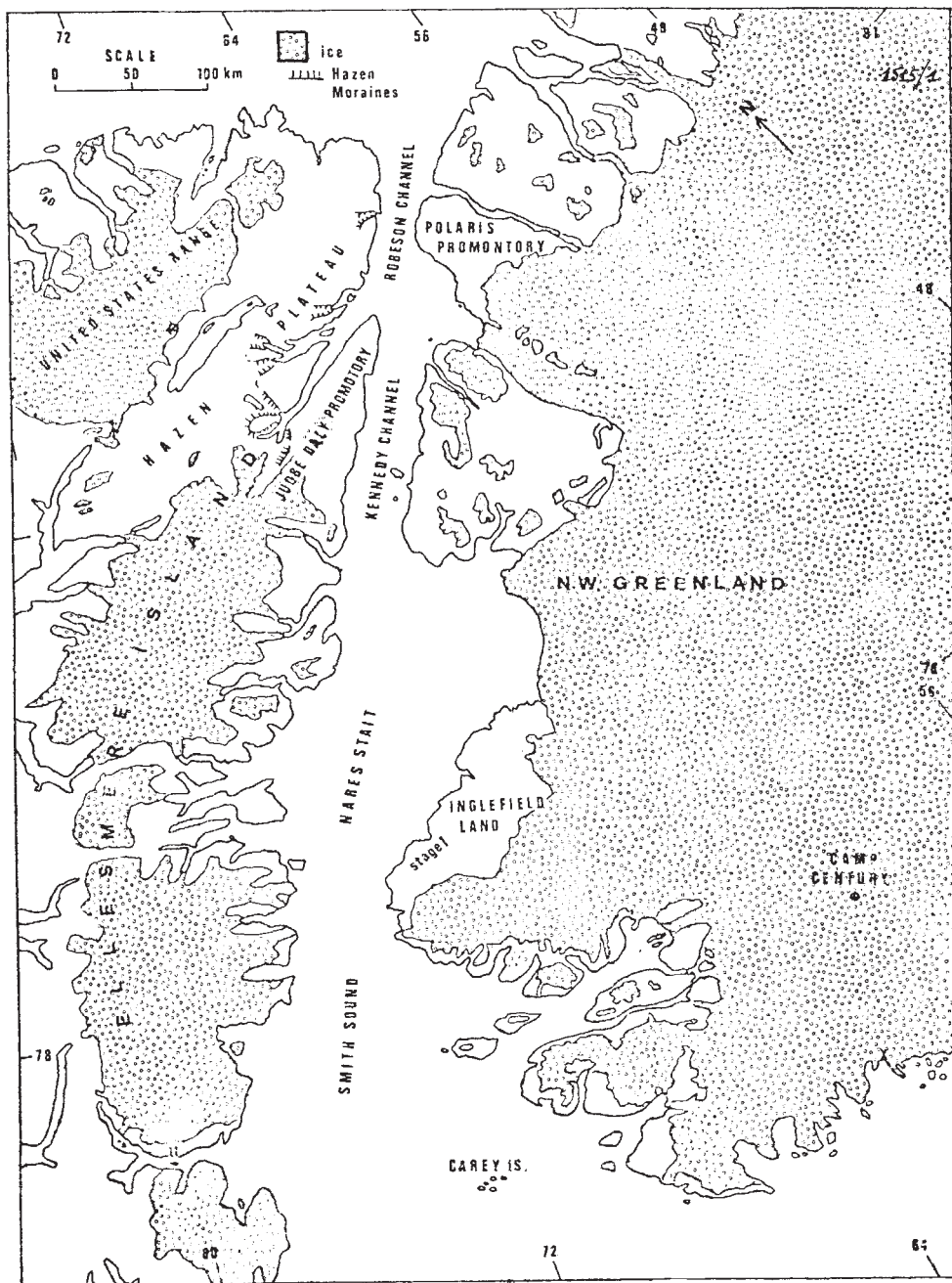


Fig. 1 Map of area covered by the Greenland Ice Sheet.

last one or two major glaciations. The restricted position of the Hazen Moraines, and the preservation of the features from the Defosse Glaciation on north-eastern Ellesmere Island, supports that suggestion.

Bendix-Almgreen *et al*¹², working on the Carey Islands, 110 km off the coast of north-western Greenland (Fig. 1), conclude from the distribution of erratics that the Greenland Ice Sheet formerly extended out to and beyond that area. They¹² suggest that Nares Strait was probably completely filled with the Greenland and Ellesmere Island ice sheets during the full-bodied stage of the Wisconsin. The date of deglaciation of the Carey Islands is not known although it may predate the age of shells from Saunders Island, 25 km off the coast of north-western Greenland, which date at more than 32,000 years BP (ref. 13). This date is probably a minimum age estimate on the last retreat of the Greenland Ice Sheet from Smith Sound. The available geological evidence and climatic considerations¹⁴ along Smith Sound, Nares Strait and Kennedy and Robeson channels does not support the conclusions

that towards the end of the last glaciation the north-western Greenland Ice Sheet was 1,300 m thicker than today¹⁵.

High coastal marine limits (> 120 m) have also been interpreted as evidence for an extensive ice advance in that region during the last glaciation. The interpretation is, however, based on the assumptions that the marine limits are all post-glacial features and that all these features represent a glacio-isostatic response related to a very extensive ice advance. As yet, however, there are no radiocarbon dates on these high coastal marine limits and the observed postglacial uplift over north-eastern Ellesmere Island and north-western Greenland can be reproduced by an uplift model¹⁶ using the restricted ice margins discussed in both areas^{5,10}.

Present evidence indicates that detailed records of multiple glaciations and sealevel changes are preserved along the coastlines of Smith Sound, Nares Strait and Kennedy and Robeson channels. There should, therefore, be stratigraphic evidence on the magnitude and age of the proposed advance of the Greenland Ice Sheet on to north-eastern Ellesmere Island

as indicated by the preservation of the oldest, subsequent ice advances in both areas. The same information applies to the stratigraphic and chronological clarification of the proposed Greenland–Ellesmere Island ice-ridge over Kane Basin.

At present the advance of the Greenland Ice Sheet on to north-eastern Ellesmere Island is considered inconclusive and, if it did occur, it would have to pre-date the late Wisconsin, and possibly the entire Wisconsin glaciation. The possible presence of Wisconsin refugia on northern Ellesmere Island¹⁷ is considered consistent with the geological evidence¹⁰.

Evidence from glacial geology is also critical for the reconstruction of the time profiles through existing and future ice cores in the region as such data will affect directly the parameters in the flow models chosen. If the ice sheets in the High Canadian and Greenland Arctic were relatively inactive during the last glaciation or glaciations then the ice cores obtained from these areas may be older than originally expected. Sections in the ice cores¹⁸ that indicate conditions colder than the present may coincide with glacial inactivity which resulted from a more continental climate, whereas the warmer sections in the cores may reflect more maritime (glacial) conditions. The chronology of glacial activity during the late Quaternary is, therefore, an important source for both the dating and palaeoclimatic interpretation of the ice cores.

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Exfoliated pebbles and sheeting in the Triassic

SHATTERED pebbles and sheeted bedrock are common weathering phenomena in most modern deserts^{1–3} but their existence in ancient desert environments does not seem to have been described. This communication documents their occurrence in South Wales, where they were formed during the Triassic period, some 200 Myr ago.

Two facies are developed in the Upper Triassic of South Wales^{4,5}—the Keuper Marl, a purplish-red dolomitic silt, which is considered to have formed in a hypersaline lake or inland sea^{6–8}, and the 'littoral' or marginal Triassic (wherein the weathering phenomena occur), red beds of variable lithology and facies, which occur below and laterally equivalent to the Keuper Marl. Environments and landforms represented by the marginal facies in Glamorgan include alluvial fans⁹ and plains, pediments, wadis¹⁰, playas and soils. The deposits of these environments (fanglomerates, calcarenites, playa silts, lacustrine cryptalgal laminites, fenestral (birdseye) limestones and calcretes) accumulated against and below a retreating Palaeozoic mountain front and around monadnocks ('islands'^{11,12}) mainly composed of Carboniferous Limestone.

Many limestone pebbles on the surface of a conglomerate, exposed at Hayes Point near Barry (Grid Ref. ST143674) are completely shattered (Fig. 1). Some pebbles have fractured to a perfect onion-skin pattern, consisting of concentric spheres of rock, split a few millimetres apart. Other pebbles have simply shattered into several angular slices. Cracks in pebbles are infilled with red silt and sand, except where the cracks have penetrated a few centimetres below the former land surface, when they are now filled with calcite. The conglomerate (110 cm thick) is a moderately sorted mixture of limestone pebbles and sand, with an irregular and deeply scoured base. It was clearly deposited relatively quickly (hours or days) and is probably the product of stream flooding. A prolonged period of weathering followed deposition of the conglomerate, when the surface pebbles were shattered and exfoliated.

At Bendrick Rock (Grid Ref. ST131668) the Triassic red beds abut against and then overlie Carboniferous limestone which must have formed a small monadnock or knoll, rising out of

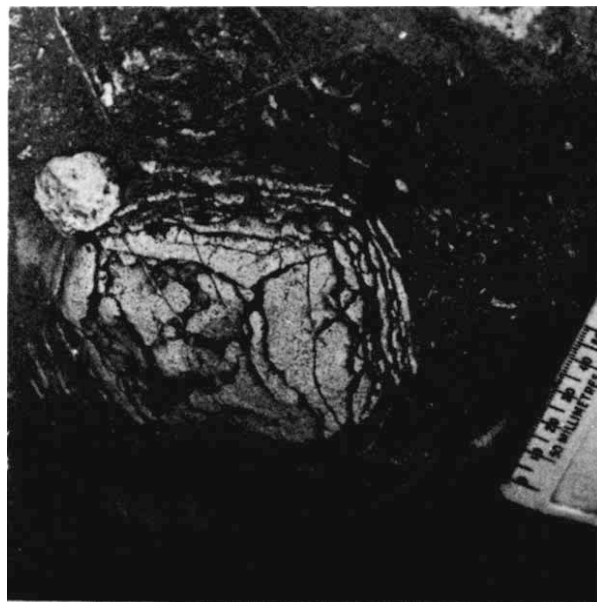


Fig 1 Exfoliated pebble of Carboniferous limestone, with concentric (onion-skin) fractures, in red Triassic sandy dolomite. Hayes Point, Glamorgan.

the Triassic pediment. The surface of the Carboniferous limestone, down to 30 cm, is split into thin sheets, parallel to the former surface. Red silt, probably of aeolian origin, occurs between the sheets. Fossils and oolites in the limestone are truncated by the cracks. The sheeting clearly developed upon the former surface and not within a weathering profile. Other features at and just below this weathered surface include neptunian dykes and an iron-staining pattern around joint blocks in the Carboniferous limestone. Calcretes (of which at least 12 occur) interbedded with red marls are developed against the Carboniferous limestone monadnock on which the sheeting is developed, and probably formed at the same time. Calcretes (pedogenic limestones) represent periods of soil formation which lasted the order of 5,000 to 30,000 yr¹¹. This suggests that the sheeting of the bedrock surface developed during a considerably longer period of time (10^4 to 10^5 yr).

Much debate has been concerned with the origin of shattered pebbles and exfoliated surfaces in modern deserts^{1,3}. Experiments by Blackwelder^{12,13} and Griggs¹⁴ demonstrated that a purely physical process of repeated temperature changes did not result in rock disintegration, but that some degree of chemical weathering (even just the presence of water) was required. Records^{15,16} of fractured pebbles of nearly chemically inert flint and quartzite do, however, suggest that insolation alone can be effective. The occurrence of frost-weathering in deserts has been realised only recently and could also be an effective cause of rock shattering, especially in higher altitude deserts, during cooler climatic phases^{1,17}. Crystallisation of salts within confined spaces¹⁸ (such as cracks along joint, cleavage and bedding planes) leads to rock disintegration. This is a common phenomenon in arid (and coastal) environments^{19,20}, particularly on saline playas, along alluvial fan channels²¹ and on high tidal flats²². Precipitation of calcite during surface calcification can have a similar rock-splitting effect²³.

In these Triassic examples of exfoliation, there is no evidence of contemporaneous chemical weathering, such as karst and solution structures, although pebbles and rock sheets are commonly dolomitised (probably a post-Triassic diagenetic event). No evidence has been found for the presence or former existence of salt deposits or their replacements at the exfoliated horizons, although salt efflorescences could conceivably have been dissolved following deposition of the overlying beds. Evaporites do occur higher in the succession (some 100 m above) towards the top of the Keuper Marl. Salt weathering, however, seems to produce linear fractures and flakes of rock^{21,22} rather than the concentric type of fracture illustrated in Fig. 1. Calcite of calcrete (pedogenic) origin does not occur within cracks—these are filled with clastic sediment or sparite druse. It would seem then that the exfoliation structures formed primarily through isolation, although water (from precipitation) or dew²⁴ may have been a contributory factor. In view of the Rhaetic marine transgression towards the end of the Triassic period, it is likely that this part of South Wales was a low altitude desert area during this time, where frost action would rarely occur.

The presence of exfoliated pebbles and sheeted bedrock is evidence additional to that provided by aeolian dune-bedding, millet-seed sands, ventifacts, calcretes and evaporites for an arid or semi-arid climate during the Triassic period in Britain. Paleomagnetic data support this deduction by indicating a low palaeolatitude (10–30 °N) for Britain during the Triassic²⁵.

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Diverse microfossils in Precambrian Onverwacht group rocks of South Africa

We report the discovery of some complex microfossils from the Kromberg formation of the Onverwacht group of South Africa. The samples, in which the microfossils were found, were collected by D.O.H. along the banks of the Komati River in the Barberton Mountain Land, at Skaapbrug, near the old JCI mining camp. The Kromberg formation lies in the Upper part of the Onverwacht group, above the Middle Marker Horizon, which has been dated as 3.355×10^9 yr old¹. The Onverwacht group is the oldest member of the Swaziland Supergroup and lies below the 3.1×10^9 yr old Fig Tree group. Microfossils have been reported from the Fig Tree group^{2,3}, and simple spherical and filamentous forms from the Onverwacht⁴⁻⁶. Spherical and cup-shaped carbonaceous microstructures have been reported⁷⁻⁹ from the Onverwacht but were not regarded as biogenic in origin.

The present forms are more complex than those previously described⁴⁻⁶; they were discovered in petrological thin sections and include spheroids, filaments and agglomerations of cells. Although spheroids have previously been described, those from the Kromberg formation⁵ fell within a narrow size range 15–20 μ m (Fig. 1a). In the present samples, more cells of the same size have been discovered but smaller cells ranging in diameter from 3 μ m to 7 μ m have also been found (Fig. 1b), having a relatively thick wall (0.5–1 μ m thick) and often black or very dark brown in colour. Although mainly found solitary and not associated with other organic matter, they are occasionally paired.

The filaments in the thin sections are of three types: (i) filaments similar in dimensions and morphology to those previously described⁵; (ii) small (diameter 1–3 μ m, length up to 25 μ m; Fig. 1c), non-septate filaments; (iii) segmented filaments (diameter 1.5–4 μ m, length up to 42 μ m, and with individual cells of diameter 1.5–4 μ m and length 2–5 μ m; Fig. 1d).

Large numbers of cells of uniform size (diameter 3–8 μ m) occur associated, set in a matrix of amorphous organic matter, on average 5–10 μ m apart (Fig. 1e).

In two samples, very rich in organic matter, we found what appears to be multicellular tissue. This is composed of very large numbers of cells of more or less uniform size (7–10 μ m; Fig. 1f). These cells are approximately equidimensional and

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their overall outline is modified by the presence of adjacent cells, that is, they appear to have walls in common with adjoining cells. No distinct overall outline to these agglomerations of cells is discernable, the maximum diameters of single agglomerations ranging up to more than 200 μm . Some of the agglomerations have a rounded outline, while others enclose areas of water-clear chert, free of organic matter.

The large variety of morphologies and the number of individual specimens leads us to the conclusion that the structures observed are biogenic in origin. Their state of preserva-

tion is variable, depending on the degree of metamorphism of the sample, but allowing for the carbonisation which has taken place^{5,10}, the morphology of the fossils is distinct and can be compared with the morphologies of specimens from younger rocks¹¹⁻¹⁴. The morphologies found—spheroids, single or paired, filaments, segmented or nonsegmented, 'colonial' structures where single spheroids occur associated, set in a matrix of apparently mucilaginous material, and multicellular structures—have all been described from prokaryotic groups such as the blue-green algae, and some of the bacteria.

There is ample evidence for the presence of biological activity in Archaean rocks. Stromatolites have been described from Rhodesia¹⁵ in rocks now dated at 3.1×10^9 yr old¹⁶, and from the Canadian Shield¹⁷ in rocks more than 2.7×10^9 yr old¹⁸. Schopf *et al.*¹⁵ believe that the discovery of the Rhodesian stromatolites indicates the activity of blue-green algae but stromatolites have been described from hot springs^{19,20} in which photosynthetic bacteria play an important role. Doemel and Brock²¹ were able to demonstrate that in the hot spring deposits at Yellowstone National Park photosynthetic bacteria control the lamination of the mat structure as a response to light fluctuations. The organism present is *Chloroflexus*, a filamentous photosynthetic bacterium which acts as a sediment trapper; it produces a laminated siliceous sediment in which the laminae are of variable thickness and where, under the regions rich in organic material, laminated silica is preserved. In the modern sediment, blue-green algae are also present to varying extents depending on the depth of the layers where light intensity may be the controlling factor. *Chloroflexus* cells are much more resistant to degradation than blue-green algae and bacterial remains are the most abundant. Although the blue-green algae virtually disappeared at low levels in the mat, however, they could still have played a role in its formation. Their results²¹ "show that stromatolites might have been formed solely by filamentous photosynthetic bacteria".

All the Onverwacht cherts studied from the Kromberg formation are finely banded with bands rich in organic material alternating with those which are poor in such. This forms the bulk of the rock studied, even when no structurally preservable microfossils can be observed. Such banded rocks are generally the result of biological activity, whether they be bacterial or algal mat structures, or glacial varves.

Thus, although the present discovery of microfossils supports the hypothesis that quite diverse remains of biological activity can be detected in the rocks of the Onverwacht group, there is at present no way of determining their biological affinities.

In light of recent work with photosynthetic bacterial stromatolites and mat structures it is possible that the microfossils we are examining are the remains of a similar type of mixture of organisms in the modern laminated mats.

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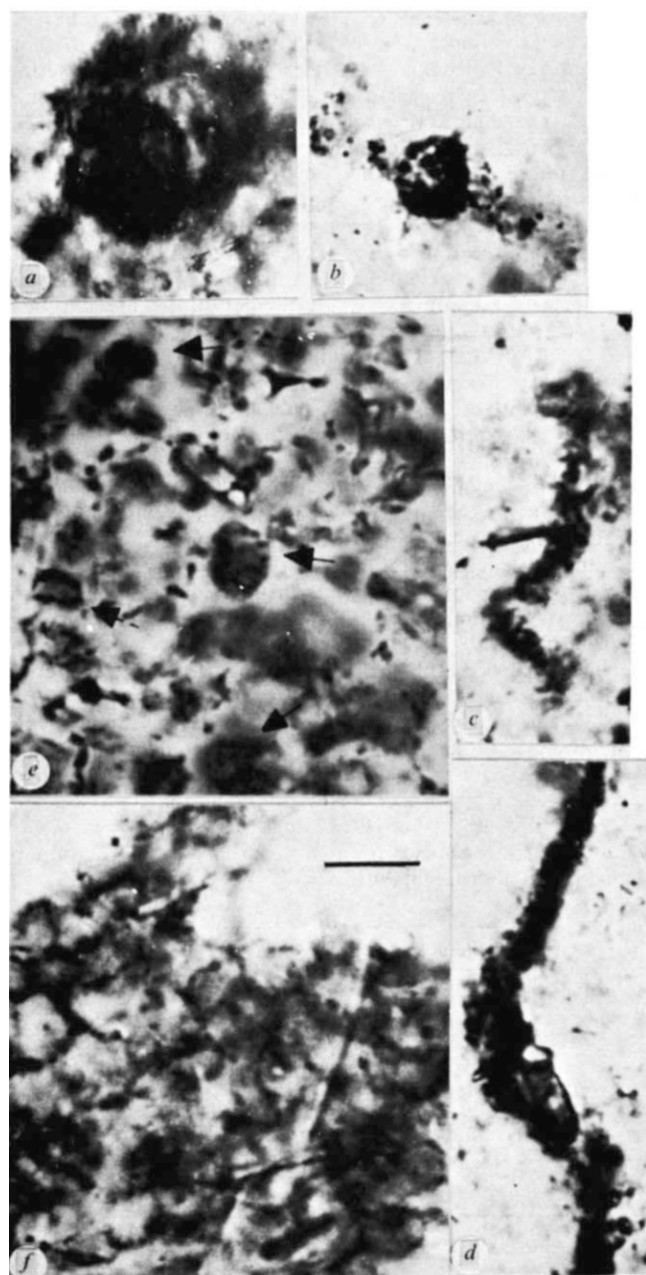


Fig. 1 *a*, Large spheroidal cell, diameter 15 μm (sample 1; thin black chert from between pillows in lava). *b*, Small spheroidal cell, diameter 6 μm (sample 7; black chert). *c*, Small nonseptate filament, diameter 2 μm (sample 1A; black chert). *d*, Segmented filament, diameter 2 μm (sample 3; black chert, very rich in organic matter). *e*, Spheres (arrowed) set in matrix of amorphous organic matter, average diameter 4 μm (sample 1A; black chert). *f*, Agglomeration of cells, diameter 7 μm , having appearance of multicellular structure (sample 3; black chert, very rich in organic matter). Scale bar represents approximately 5 μm (all micrographs).

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Reliability of amino acid racemisation dating and palaeotemperature analysis on bones

THE general inability of isotope geologists to work out techniques for dating continental Pleistocene deposits has led to the conception of nonisotopic chemical methods. Hare and Mitterer¹ noted that fossils could possibly be dated by determining the extent to which the *l* optical isomer of a given amino acid had racemised to form the *d* isomer which is initially absent in skeletal material. Obviously, the rate constant for the reaction must be known accurately if age is to be calculated, but its value is difficult to assess: it is highly temperature dependent² (about 20% °C⁻¹); it depends on the state of the amino acids (free amino acids racemise about an order of magnitude more rapidly than those bound as peptides)^{3–5}; and it is highly dependent on environment⁶. Furthermore, free amino acids may back react to produce the bound forms, and there could be open system conditions during diagenesis⁸.

Bada *et al.* have investigated amino acid racemisation geochemistry as a method of dating fossil bones² and as a means of deducing palaeotemperature data where ages have been independently determined. They showed that 'ages' or 'palaeotemperatures' calculated from data of racemisation of amino acids in Upper Pleistocene bones are generally comparable to independently deduced values. That does support the notion that racemisation may be useful in geochronological and palaeoclimatic evaluations, but their results have many inconsistencies.

Bada *et al.*² determined the extents of racemisation of amino acids in two or three year old bone fragments collected in the Arizona desert. From the observed extent of racemisation of glutamic acid, and the temperature dependence of the rate constants, it is possible to calculate that the sample was preserved at an average temperature of about 75° C. That is however, completely unlikely. A more reasonable explanation for the anomalously large degree of racemisation is that there is a brief interval after the death of the organism, during which racemisation proceeds more rapidly than it does later in the history of the fossil. If that is actually the case then 'ages' or 'temperatures' which are calculated by assuming constant racemisation, may be quite misleading.

Moreover, the rate constant, normalised to a given temperature, is extremely sensitive to the environment. In particular, the extent of isoleucine racemisation (actually epimerisation) in bone from La Brea tar pits was found to be much less than expected from data on isoleucine racemisation on samples from Muleta Cave and elsewhere⁶. This is apparently because of the relatively anhydrous environment provided by the asphalt in which the La Brea sample was preserved.

Turekian and Bada⁷ have established a chronological sequence in a section from Muleta Cave in Mallorca from radiometric and isoleucine racemisation ages (the latter were calculated by assuming that the temperature there has been constant at 19° C and that the rate constant at this temperature is $6 \times 10^{-6} \text{ yr}^{-1}$). Their results show that amino acid dates and ²³⁰Th dates agree (the ¹⁴C dates are much younger, but that is probably because whole bone was analysed, rather than just the collagen fraction⁸). Bada⁹ has redetermined the rate constant, and his recent results give a value of $2.6 \times 10^{-6} \text{ yr}^{-1}$ at 19° C (this value is also similar to that estimated using the rate constant for aspartic acid, calculated for the 8,600 yr old Muleta sample⁷, and the ratio of rate constants for aspartic acid and isoleucine racemisation³). Ages recalculated using this value of *k* are lower than previously calculated racemisation ages by more than a factor of two, and are inconsistent with ²³⁰Th/U ages (Table 1).

Bada has also established a chronology from the Z section of Muleta Cave (Table 2). It is clear from these results that the ¹⁴C ages on bone collagen fraction, the amino acid chronology, and the ²³⁰Th chronology are all strongly discordant. This apparent discordance becomes even greater when *k* is modified to account for the facts that temperatures were generally lower than 19° C and that it was the collagen fraction, which racemises more slowly than the whole bone¹⁰, which was sometimes analysed.

Table 1 Calculated age sequence from X sector in Muleta Cave*

Position in section (cm)	Nature of sample	Dating technique	Calculated age (yr)
80	Travertine	²³⁰ Th	85,000 and 97,000
95	Collagen fraction of bone	Isoleucine racemisation	180,000
200	Collagen fraction of bone	Isoleucine racemisation	280,000
250	Bone	Aspartic acid racemisation	69,000
300	Whole bone	¹⁴ C	16,400
350–400	Whole bone	¹⁴ C	18,735

* Isoleucine racemisation ages are calculated using $k = 2.6 \times 10^{-6} \text{ yr}^{-1}$.

Table 2 Calculated age sequence from section Z in Muleta Cave*

Position of sample in section (cm)	Nature of sample	Dating technique	Calculated age (yr)
350	Bone	¹⁴ C	14,500
350	Bone	Isoleucine racemisation	32,000
475	Bone	Isoleucine racemisation	26,000
550	Collagen fraction of bone	Isoleucine racemisation	120,000
550	Bone	Aspartic acid racemisation	34,000
550–600	Collagen fraction of bone	¹⁴ C	28,600

* Using $k = 2.6 \times 10^{-6} \text{ yr}^{-1}$.

Table 3 Ratios of racemisation rate constants for amino acids*

	Isoleucine Leucine	Glutamic Leucine	Alanine Glutamic	Aspartic Leucine
Predicted by heating experiments ²	1	2-2.4	1	8
Muleta Cave (28,900 yr, ¹⁴ C age)	0.90±0.2	3.0±0.6	0.84±0.2	5.8±1.0
Florisbad (38,600 yr, ¹⁴ C age)	1.3±0.2	1.8±0.4	1.6±0.3	7.3±1.0
Border Cave (>45,000 yr, ¹⁴ C age)	0.84±0.2	2.5±0.5	1.2±0.2	4.8±1.0
Olduvai (~200,000-300,000 yr)	1.1±0.2	2.1±0.4	0.92±0.2	2.5±0.5
Swartkyans (~2 Myr)	1.6±0.3	1.3±0.3	1.3±0.3	2.6±0.5

* Calculated from the results of Bada *et al.*², k by assuming the d isomer to be absent in modern specimens. Errors in the rate constant ratios are estimated at $\pm 20\%$ using the analytical uncertainty ($\pm 10\%$) in the gas chromatographic analyses of the d/l ratio (K. A. Kvenvolden, personal communication).

Schroeder and Bada¹⁰ determined the extent of aspartic acid racemisation in ¹⁴C dated bones (see also ref. 2), and used the results to calculate palaeotemperature histories. Using reasonable assumptions they calculated that the temperature in Muleta Cave was about 4°C colder between 10,000 and 18,000 years ago than it is at present, a conclusion consistent with most other evidence. Using their data and approach to calculate the average Muleta temperature between 19,000 and 29,000 yr BP (sample 1704A) it is possible to estimate that temperatures during that period were similar to those at present. Most other evidence, including that summarised by Schroeder and Bada, indicates, however, that during that period the temperature was much cooler than at present. The difference can be reconciled if it is assumed that the ¹⁴C age is wrong, but such an assertion would undermine other conclusions.

Bada *et al.*² determined rates of racemisation of amino acids in bones heated at elevated temperatures in laboratory experiments. They pointed out that if a given sample has not been altered by open system conditions, then the relative extent of racemisation of the different amino acids must be consistent with the relative extents predicted from the rate constant data. That is an important observation because it allows a check on whether or not the sample has been a closed system. The check can be applied by using the ratios of optical isomers for the various samples to calculate ratios of rate constants for the various amino acids, so that the results can be compared with those predicted from the high temperature heating experiments of Bada *et al.*² (Table 3).

For all samples at least one of the calculated rate constant ratios is inconsistent with the predicted value (Table 3), so all of these samples have suffered some contamination. Different samples seem to be contaminated with different amino acids. In my opinion no meaningful conclusions of palaeotemperature or palaeoclimate can be deduced from any of these results.

The contribution from Bada *et al.*² is very significant in that it outlines quantitative, *a priori*, criteria for determining if amino acid racemisation ages or temperatures are reliable. Their findings, and the fact that reasonable ages and temperatures are sometimes obtained, indicates that the method has potential. It clearly faces many basic problems, however, and in my opinion no palaeoclimatic or geochronological inferences should be drawn from racemisation data until the basic geochemistry is thoroughly understood and the bases of the method firmly established.

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DR BADA REPLIES—Bender's review of my work is both inaccurate and incomplete. He has not cited two of my publications dealing with aspartic acid racemisation dating^{1,2}. (Although one paper was only recently published, I sent Bender a preprint the first of this year when he informed me he was writing a review.) In those articles I show that after 'calibrating' the amino acid racemisation reactions using a radiocarbon dated bone, it is then possible to date other bones from the same site, which are either too old or too small for radiocarbon dating. The only assumption in this approach is that the average temperature experienced by the calibration sample is representative of the average temperature experienced by the other sample. Ages thus deduced are in good agreement with radiocarbon ages determined on the same samples².

Bender points out that several factors (temperature, protein hydrolysis, and so on) can influence the extent of amino acid racemisation in a fossil. I am fully aware of these factors and have discussed the potential limitations of racemisation dating which arise from each of them³⁻⁵.

The bovine bones discussed by Bender were found lying on the surface in the desert plateau region of south-western Arizona³. They were collected because I wanted modern bone samples for kinetic studies of isoleucine epimerisation in bone. The samples seemed ideal, as they had already experienced the early phase of animal bone diagenesis (that is, carnivore and microbial consumption of the adhering flesh). The low alloisoleucine/isoleucine ratio (0.02) indicated the bones were not very old.

Following the isoleucine studies, an analysis of the extent of racemisation of aspartic acid in the desert bones was carried out, and this indicated a d/l ratio of 0.15 (ref. 4). An analysis of a piece of bone from a beef steak, however, yielded a d/l aspartic acid ratio of 0.07 (ref. 1). That value is in close agreement with d/l ratios of aspartic acid found for several proteins carried through the same analytical steps as the bone samples^{6,7}. A d/l ratio of 0.07 must, therefore, represent the $t = 0$ value for aspartic acid.

The aspartic acid results suggested that the desert bones were probably older than the 2-3 yr originally estimated. To calculate an average temperature to which the bones have been exposed, as Bender has done, in the absence of firm documentation of their age, is unwarranted. Furthermore, although the present mean temperature in the region where the bones were found (Yuma, Arizona) is 22°C (ref. 8) ground temperatures as high as 80-85°C have been reported (M. A. Barker, unpublished). An older age and the occasional ex-

tremely high temperatures could account for the extent of racemisation in the Arizona desert bones.

Bender contends that racemisation rates in bone are extremely sensitive to environmental factors other than temperature. He states that Schroeder and myself⁹ also noticed this; we have never made this statement and would, in fact, strongly disagree with it. The rate constants of aspartic acid racemisation determined from the extent of racemisation in radiocarbon dated bones younger than 10,000 yr (post-glacial), all show a remarkable correlation with the present mean temperatures of the general regions in which the bones were found^{1,2,9,10}. The same correlation exists for glacial age bones, but this should be considered less reliable, as temperature changes during the last ice age may have been different at the various sites. The sites we have studied range from temperate coastal to near tropical mountainous regions in Africa, and from hot, arid rock shelters in Iran and Iraq to a limestone cave on Mallorca. If environmental factors other than temperature had an important influence on racemisation rates, it would seem that the correlation between the rate constants and the average temperatures for these various sites would not be as good as it is.

The racemisation rates^{4,11} for the bones from the La Brea Tar Pits are indeed anomalous. This type of environment is, however, rare and extreme. Racemisation rates determined from radiocarbon dated bones from the surrounding southern California area would hardly be expected to be applicable to the tar pits. In order to date the bones in the tar pits, a 'calibration' should be carried out by determining the extent of racemisation in a radiocarbon dated bone from the tar pits, and then other samples from the tar pits could be dated.

The isoleucine ages published by Turekian and myself for Muleta Cave¹² were only preliminary results, to show the possible potential of the racemisation technique. Detailed kinetic studies at elevated temperatures have yielded better estimates of k_{iso} at the Muleta Cave temperature³, and these k_{iso} values have yielded ages in reasonable agreement with radiocarbon ages^{3,4}. Also, the allo/iso ratios in the Muleta Cave bones were very small (less than 0.1) and difficult to measure accurately. The isoleucine ages were only rough estimates.

We have obtained aspartic acid racemisation ages for Muleta Cave (ref. 2, and J. L. Bada, unpublished) (Table 1). When the radiocarbon age is based on the collagen component, it agrees excellently with the aspartic acid racemisation age. The racemisation age determined for the bone from the X section is, however, much older than the radiocarbon ages obtained from that section. This is not surprising, as the radiocarbon ages were based on the total acid-leached carbon.

Table 1 Muleta Cave ages

Cave section*	d/l Aspartic acid	Aspartic acid age† (yr)	¹⁴ C age (yr)
Z-400 cm (UCLA 1704D)‡	0.273		16,850 ± 200
E-350 cm (UCLA 1704E)	0.293	18,600	18,980 ± 200
Z-550-600 cm (UCLA 1704A)	0.455	33,700	28,600 ± 600
X-250 cm (SI-648)	0.73	69,000	16,335 ± 415
X-350 to 400 cm (SI-650)			18,735 ± 555

* Notation in parentheses is radiocarbon laboratory identification number. The UCLA results are collagen dates, whereas the SI ages are for acid leached carbonates.

† $k = 1.25 \times 10^{-5} \text{ yr}^{-1}$.

‡ First sample used for site calibration (see refs 1 and 2). Aspartic acid racemisation results taken from ref. 2 and J. L. Bada (unpublished).

Such ages are often unreliable because of exchange processes involving ground water carbonates of different specific activities¹³.

The aspartic acid age for bone from the 250 cm horizon of the cave X section suggests an age of about 100,000 yr at its base (the total section depth is approximately 400 cm). The ²³⁰Th age of the surface of a stalagmite around which the section formed was found to be $85,000 \pm 8,000$ yr (ref. 12). This age is consistent with the age of 100,000 yr for the base of the section, as it seems likely that carbonate accumulation on the stalagmite would stop shortly after sedimentation in the section began.

Schroeder and I⁹ did not use sample 1704A in our temperature calculations because of the uncertainties in the temperature model for the Mediterranean area at 20,000–30,000 yr BP. Also, the radiocarbon age of this sample is probably less reliable than that of the other radiocarbon-dated samples from Muleta Cave, as it was collected from an horizon 50 cm thick whereas the other samples were obtained from more discrete horizons. Thus, the radiocarbon age represents an 'average' over the 50-cm section. The racemisation analysis was carried out on a single 10 g piece which could have an age slightly different from the 'average' radiocarbon age.

Using the procedure outlined by Schroeder and myself⁹, I calculate that the difference between the average temperatures experienced by samples 1704C and 1702A (see Table 1) is -0.8°C , compared with the difference of -1.6°C experienced between 1704C and 1704D. Evidence^{9,14} indicates that there was a period of gradually increasing temperature between ~20,000 and 50,000 yr BP, although temperatures never reached present values. Some of the coldest temperatures during the last glacial occurred at about 15,000–20,000 yr BP (ref. 14). Therefore, a bone of about that age (such as 1704D) would yield a slightly cooler average temperature than a bone with an age of around 30,000 yr (such as 1704A).

Because the relative rates of amino acid racemisation determined by heating modern bone fragments at elevated temperatures are not exactly the same as those in fossil bones, Bender believes that all fossil bones analysed by Kvenvolden, Peterson and myself⁴ were contaminated to some degree with modern amino acids. Although the Arrhenius activation energies (E_a) are about the same for the various amino acid racemisation reactions, they certainly differ by at least tenths of a kcalorie per mol (the experimental uncertainties of the E_a values). This difference is not enough to change the order of racemisation rates (that is, aspartic acid > alanine $\approx$ glutamic acid > leucine $\approx$ isoleucine) in going from elevated to lower temperatures, although it could cause small changes in the relative rates. For example, if any two reactions have E_a values of 33.0 and 32.0 kcalorie mol⁻¹ and the ratio of the rate constants is 5:1 at 100°C, then at 20°C the ratio would have changed to 3.5:1. Thus, the relative rates of racemisation in uncontaminated fossil bones could be different from those found in modern bones heated at elevated temperatures.

Bender (his Table 3) shows that the relative racemisation rates for bones from Muleta and Border Caves are essentially identical (within experimental error). As these two bones differ considerably in age and in depositional environment, I suggest that this similarity, in fact, indicates that these bones are not contaminated and that the relative rates of racemisation observed represent the pattern expected in uncontaminated bones. Other samples in Bender's Table 3 are contaminated to various degrees, a point which I have emphasised before⁴.

Bender seems to have missed the point that even if a bone is contaminated, racemisation can still provide a minimum age. Contamination introduces amino acids that are more modern (that is, consist of mainly the *L*-enantiomers) than the indigenous collagen-bound amino acids, and therefore lowers the d/l amino acid ratios.

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Theory of liquid-liquid and liquid-vapour equilibria

WE formulate here a general molecular theory of solutions, which predicts a first order phase transition (boiling), volume changes on mixing, and the complete phase behaviour of pure and mixed fluids. Our formulation is based on a simple hole theory of the liquid state.

The total change in the Gibbs free energy that is associated with the creation of N_o holes in a liquid of N_l molecules of size r , is given by¹

$$\Delta G = v_l N_o E_h + P N_o v_h + RT [N_o \ln v_o + N_l \ln v_l] \quad (1a)$$

or, in reduced variables, by

$$\begin{aligned} \Delta G / r N_l E_h &= G - G^0 \\ &= (1 - 1/v) + P(v - 1) + T[(v - 1) \ln(1 - 1/v) \\ &\quad + (1/r) \ln(1/v)] \end{aligned} \quad (1b)$$

where, r = hard core molecular volume/hole volume, v_h ; $v_o = 1 - v_l = N_o / (N_o + r N_l)$ = volume fraction of holes; ϵ = intermolecular interaction (contact) energy; $E_h = \gamma \epsilon / 2$ = hole energy; γ = lattice coordination number; $P = P v_h / E_h$ = reduced pressure; $T = RT / E_h$ = reduced temperature; V = macroscopic volume; $V_o = r N_l v_h$ = hard core volume of molecules; $v = V / V_o = 1 / v_l$ = reduced volume; $G^0 = -1 + P$ = reduced free energy of liquid without holes.

Equation (1) can be obtained directly from a lattice model in which the partition function is evaluated in the mean field approximation. It can also be obtained without appealing to a lattice description of the liquid state¹.

The equilibrium number of holes can be determined by minimising the free energy with respect to N_o , or equivalently, the reduced volume v . Thus:

$$(\partial G / \partial v)_{T, P, N_l} = 1/v^2 + P + T[\ln(1 - 1/v) + (1 - 1/r)(1/v)] = 0 \quad (2a)$$

or

$$\begin{aligned} 1/v &= f(1/v) \\ &= 1 - \exp[-1/(T v^2) - (1 - 1/r)(1/v) - P/T] \end{aligned} \quad (2b)$$

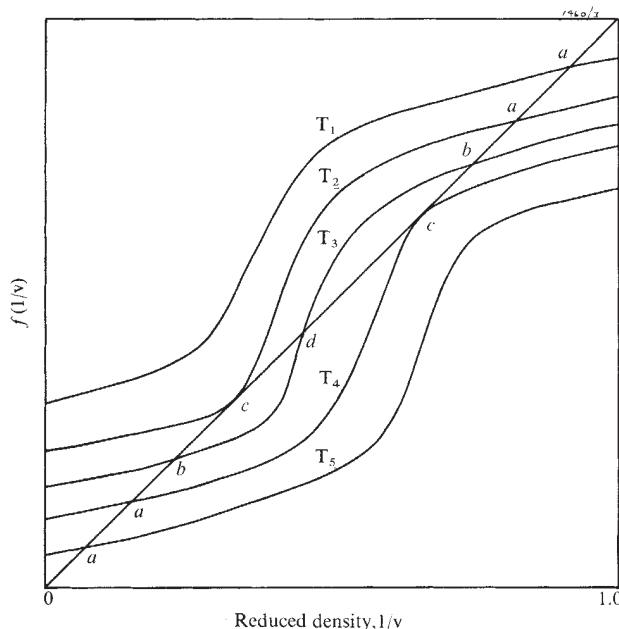


Fig. 1 Schematic representation of the graphical solution of the equation of state, equation (2b), below the critical point. The pressure is held constant and the temperature is varied. The various solutions to the equation of state produce the following kinds of behaviour in the Gibbs free energy, G : points a , absolute minima; points b , relative minima; points c , inflection points; points d , relative maxima. $T_1 < T_2 \dots < T_5$.

The equation of state (2a or b) coupled with equation (1) defines the Gibbs potential in terms of its natural variables, T and P . Thus, all thermodynamic properties of the model are determined. Note that equation (2) is to be interpreted as defining the volume v in terms of T and P . This is an important distinction which assures that G will always be defined in terms of its canonical variables T and P ; this further ensures that thermodynamic oddities, such as negative pressures, or compressibilities, will be avoided.

Equation (2b) can be solved graphically (Fig. 1). The pressure is held constant and the temperature is varied. At the lowest temperature, T_1 , only one solution to equation (2) exists (point a) and it corresponds to a high density phase (liquid). Point a corresponds to an absolute minimum in G . As the temperature is increased to T_2 , a second solution appears at the tangent, point c , which corresponds to a low density phase (gas). A corresponding inflection point occurs in G at point c whereas an absolute minimum in G still occurs at the new point a . At a temperature T_3 slightly above T_2 , there are three solutions to equation (2): two of them (points b) correspond to relative minima in G and the third (point d) yields a relative maximum in G . Thus, at temperatures near T_3 both liquid and gas phases are possible and, in general, one phase will be metastable with respect to the other. At T_4 the last vestige of a liquid phase is seen (metastable limit for liquid phase), and by T_5 only a low density gas phase is present. There is a unique temperature, near T_3 at which the free energy minima will be equal; this is the equilibrium saturation temperature. In the pressure-volume plane the locus of all such points define the classical saturation curve (binodal) (Fig. 2), and the locus of all the tangent points c defines a metastability limit (spinodal) (Fig. 2).

The spinodal is determined from $\partial f(1/v) / \partial (1/v) = 1$ and equation (2) which defines the tangent points c (Fig. 1), and yields

$$P_s = - \frac{2v_s(v_s - 1) \ln(1 - 1/v_s) + (1 - 1/r)(v_s - 1) + v_s}{v_s^2[v_s - (1 - 1/r)(v_s - 1)]} \quad (3)$$

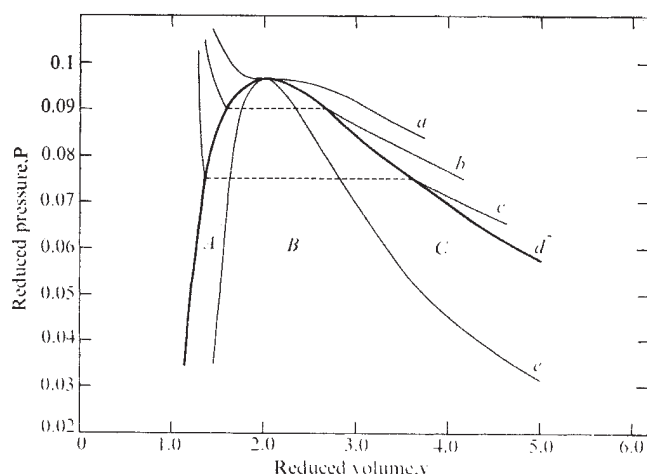


Fig. 2 Theoretical Pv diagram of a fluid for which the ratio of the hard core volume of the molecule to the hole volume is unity ($r = 1$). Along the equilibrium saturation curve (binodal), the Gibbs free energy, G of the liquid and gas phases are equal. The metastability limit (spinodal) is determined by equation (3). The set of all P, v points in the unstable region B yield relative maxima in G ; G is nonanalytical (essential singularity) at the critical point. Tie lines connect isothermal states on the binodal, but not on the spinodal; spinodal temperatures are determined by equation (4). a , $T = 0.500$; b , $T = 0.483$; c , $T = 0.455$; d , equilibrium saturation curve; e , metastability limit. A , Metastable liquid; B , unstable; C , metastable vapour.

for $v_s^0 \leq v_s < \infty$; where, v_s and P_s are the reduced volume and pressure that define the spinodal in the Pv plane (see Fig. 2), and v_s^0 is the value of v_s for which $P_s = 0$. The reduced spinodal temperature, T_s , is given by

$$T_s = [2(1 - 1/v_s)]/[v_s - (1 - 1/r)(v_s - 1)] \quad (4)$$

The critical point not only satisfies equation (3), but also $\partial^2 f(1/v)/\partial(1/v)^2 = 0$. These conditions on $f(1/v)$ are equivalent to the usual conditions for a critical point, (P_c, v_c, T_c) , that is, $(\partial P/\partial V)_T = 0$, and $(\partial^2 P/\partial V^2)_T = 0$, and they yield:

$$v_c = 1 + \sqrt{r}; T_c = 2r/(1 + \sqrt{r})^2 \quad (5)$$

$$P_c = [2/(1 + \sqrt{r})^2][r \ln(1 + 1/\sqrt{r}) + 1/2 - \sqrt{r}] \quad (6)$$

Close examination of the equation of state shows that the free energy has an essential singularity at the critical point².

The molar critical compressibility factor, Z_c , is given by

$$\begin{aligned} Z_c &\equiv P_c v_c / RT_c \\ &\equiv r(P_c v_c / T_c) \\ &= (1 + \sqrt{r})[r \ln(1 + 1/\sqrt{r}) + 1/2 - \sqrt{r}] \end{aligned} \quad (7)$$

For $r = 2$, $Z_c = 0.375$ which is identical to that obtained from the VDW and Berthelot equation of states³. The similarity of equation (2) and the VDW equation becomes more apparent when the former is expanded in virial form:

$$\begin{aligned} PV/RT &\equiv r(Pv/T) \\ &= 1 + r(1/2 - 1/T)(1/v) + (r/3)(1/v)^2 \\ &\quad + (r/4)(1/v)^3 + \dots \end{aligned} \quad (8)$$

The temperature dependence of the second virial coefficient is identical to that of the VDW equation.

Other observations are noteworthy. First, it is often assumed in hole theories that the total hole energy, E , is proportional to the number of holes ($E = N_o E_h$). This is a valid approximation only at high densities. At low densities, hole-hole contacts become prevalent; when the random mixing correction for hole-

hole contacts is made, the energy becomes $v_1 N_o E_h$. Significantly, if the v_1 factor is ignored, no phase transition will occur. Second, the equilibrium saturation curve can be determined without appealing to the Maxwell construction or any other construction outside thermodynamics. Third, the equation of state, equation (2a), implies a generalised corresponding states theorem; that is, all molecules of size r obey the same reduced equation of state. Fourth, for a homologous series of liquids (such as the normal alkanes) the theoretical boiling point increases with increasing molecular weight, as observed experimentally.

The generalisation of equation (1) that is appropriate for a binary mixture is straightforward. Formally, the system is ternary (two types of molecules, of sizes r_1 and r_2 , plus holes). The only new parameter that is required to describe the mixture is the interaction energy ϵ_{12} between the two components. The free energy of mixing, ΔG_m , is given by

$$\Delta G_m = \bar{r}N(\bar{E}_h G_{12} - x_1 E_h^1 G_1 - x_2 E_h^2 G_2) \quad (9)$$

and

$$\begin{aligned} G_{12} &= -1/v + Pv + T[(v-1) \ln(1-1/v) + (1/r) \ln(1/v) + \\ &\quad + (x_1/r_1) \ln x_1 + (x_2/r_2) \ln x_2] \end{aligned} \quad (10)$$

where, $N = N_1 + N_2$; $\bar{r} = (r_1 N_1 + r_2 N_2)/N$; $x_1 = 1 - x_2 = r_1 N_1 / \bar{r}N$; $E_h^1 = \gamma \epsilon_{11}/2$; $E_h^2 = \gamma \epsilon_{22}/2$; $\bar{E}_h = \gamma/2(x_1^2 \epsilon_{11} + 2x_1 x_2 \epsilon_{12} + x_2^2 \epsilon_{22})$; $T = RT/\bar{E}_h$; $P = P v_h \bar{E}_h$; $v = V/V_o$; $V_o = \bar{r}N v_h$; and G_1 and G_2 are the free energies for the pure components, 1 and 2 [compare with equation (1b)]. The enthalpy, entropy, and volume changes that occur on mixing are obtained in the usual way by taking derivatives on ΔG_m . The functional form of the equation of state of the mixture is the same as that for the pure fluid, except that the reduced variables are as in equation (10), and r is replaced by $\bar{r}$. The extension of the theory to any number of components only requires the obvious generalisation of equations (9) and (10). Only in the limit $v = v_1 = v_2 = 1$ does ΔG_m reduce to the Flory-Huggins expression⁴. In the general case of zero volume change, $v = x_1 v_1 + x_2 v_2$, there are still significant contributions of v_1 and v_2 to both the entropy and enthalpy of mixing.

The chemical potential $\Delta \mu_1$ is given by:

$$\begin{aligned} \Delta \mu_1 &\equiv (\partial \Delta G_m / \partial N_1)_{P, T, N_2} \\ &= r_1(\bar{E}_h G_{12} - E_h^1 G_1 + x_2 \partial(\bar{E}_h G_{12}) / \partial x_1) \end{aligned} \quad (11)$$

and $\Delta \mu_2$ can be obtained by symmetry.

For liquid-liquid equilibria, the binodal is determined by equating chemical potentials across the liquid phases. For a non-polar polymer mixture ($r_2 \gg r_1$) where $\epsilon_{12} = (\epsilon_{11} \epsilon_{22})^{1/2}$ both upper and lower critical temperatures of solution are predicted. Theoretical, negative, excess volumes are also obtained under these conditions. Equating chemical potentials across liquid and vapour phases allows for the complete determination of the boiling point composition diagram (distillation diagram). For $r_1 = r_2 = 1$ and $\epsilon_{12} = (\epsilon_{11} \epsilon_{22})^{1/2}$, nearly ideal behaviour is obtained. Deviations of ϵ_{12} from the geometric mean, or large dissimilarities in molecular size, cause maxima or minima to occur in these diagrams; azeotropes are thus predicted. To our knowledge, no other molecular theory has been presented which predicts the entire range of distillation phenomena from a unified point of view.

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Load-bearing structures and crystal intergrowth

As far as I am aware, no one has attempted to place the concept of crystal-crystal intergrowth on a quantitative basis. The object of this note is to calculate the probability of the formation of a three-dimensional load-bearing structure on the basis of crystal-crystal intergrowth.

The origin of the load-bearing capacities of structures like inorganic cements and bone is still disputed. In each of these structures, themselves composites, the solid component, generally assumed to be the main load-bearing element, is mainly crystalline in nature, though usually of submicrometre size. Two mechanisms have been proposed to explain load-bearing capacities. In the first it is assumed that the solid particles form a physically bonded structure¹⁻³ whereas the other assumes that the solid particles form a three-dimensional structure by crystal-crystal intergrowth at the points of contact⁴⁻⁷. In either case, the points of contact or the points of intergrowth must be numerous and spread throughout the volume for the structure to be functional.

In inorganic cements as well as in bone, crystallisation begins in isolated regions. Subsequent crystallisation and crystal growth bring the crystals from different regions into contact. Thus, when two crystals from two different regions meet, they do so at a random orientation. The case of apatite crystals in long bones is somewhat complicated. The apatite crystals in long bones of a newly born baby are randomly oriented; those in mature bone, however, have a preferred orientation with the *c* axis parallel to the long axis of the bone⁸.

When two crystals from two different regions come into contact two possibilities may arise. In the first case, the atoms at the interface can be considered to belong to the crystal structures of both crystals and because of the structural continuity the intercrystalline bond is nearly as strong as the intracrystalline bond. In the second case there is no such relationship and the intercrystalline bond is much weaker than the intracrystalline bond so the crystals can be considered to be physically bonded.

For ideal crystals, crystal-crystal intergrowth can only occur when the crystals meet each other in a symmetry related indistinguishable orientation. For micrometre sized real crystals some angular relaxation of the order of the mosaic spread in a single crystal—that is 3–4°—is expected. The probability that two crystals will meet each other in this orientation, by accident, can be calculated.

Let (*hkl*) be the crystal plane across which the crystal-crystal intergrowth is going to occur. Let *c* be an axis normal to the plane having an *n* degree rotation symmetry and *a* be an axis in the plane. If a second crystal is brought into contact with the first an intergrowth will occur when both *c* and *a* axes of the two crystals are within the angular relaxation zones of each other. Statistically, the second *c* axis can approach the first from any point on a hemisphere drawn round the first. The probability of two *c* axes being within the relaxation cones of each other is given by

$$P_c = (1 - \cos 4^\circ) = 0.002$$

The *a* axis will repeat *n* times in 360°, so that when two *c* axes are parallel to each other the probability of the two *a* axes coming within each other's relaxation zones is given by

$$P_a = n \times 4^\circ / 360^\circ = n/90$$

so that the total probability of intergrowth is

$$P_t = P_c P_a = n/45,000$$

The probability of a third crystal joining either of the first two in intergrowth orientation is given by P_t^2 and so on. Thus the probability of the formation of an extended structure on this basis is vanishingly small. Even if most of the *c* axes are parallel to each other, somewhat like apatite crystals in mature bone, the probability that a load bearing structure will form will still be extremely small. The probability of obtaining an aggregate 1 mm long from micrometre-sized crystals on this basis will be $(n/90)^{999}$ where *n* is a small integer. This very low probability is consistent with a scanning electron microscopic work on plaster of Paris blocks⁹. This work did not reveal any definite evidence for the occurrence of extensive crystal-crystal intergrowth though one of the objects of the study was to search for it.

In the case of physical bonding of the crystals there is no requirement of any continuity of atomic arrangement between the participating crystals, so bonding will occur whenever crystals come sufficiently close to each other. The probability of the formation of an extensive structure on this basis depends only on the packing density of the crystals and on their size and shape¹⁰.

Thus the most probable mechanism of the origin of load-bearing capacities of bone and inorganic cements is the physical bonding of the crystals.

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Direct observation of alkali metal colloids in alkali halide crystals

SIEDENTOPF¹ observed that sodium chloride crystals containing atomically dispersed F centres turned blue and exhibited Tyndall scattering when annealed at 400° C. These effects were attributed to colloidal particles of sodium metal formed in NaCl crystals by aggregation of F centres during the annealing process. Savostianova² applied the Mie³ theory to absorption and scattering of light from colloidal metallic particles suspended in dielectric media and predicted colloid sizes in excess of 80 nm. The blue colour of natural rocksalt, investigated⁴ using optical absorption and photoconductivity, was similarly attributed to colloids. Ionising radiation also produces colloidal absorption bands in irradiated alkali halide crystals under certain conditions⁵⁻¹⁰.

Increasingly sophisticated techniques have been applied to the study of these colloidal centres, for example, ultramicro-

scopy¹¹, diffuse X-ray scattering^{12,13}, NMR^{14,15}, EPR¹⁶⁻¹⁸, electrical conductivity¹⁹ and surface replication²⁰⁻²⁶. Despite 70 years of investigation, their existence has still remained controversial, partly for the want of a direct method for observing actual colloidal particles *in situ*, and in part to disagreement over possible mechanisms of colloid formation²⁷⁻²⁹, particularly over the role of existing defects such as dislocations and impurities^{11,25,30-32}.

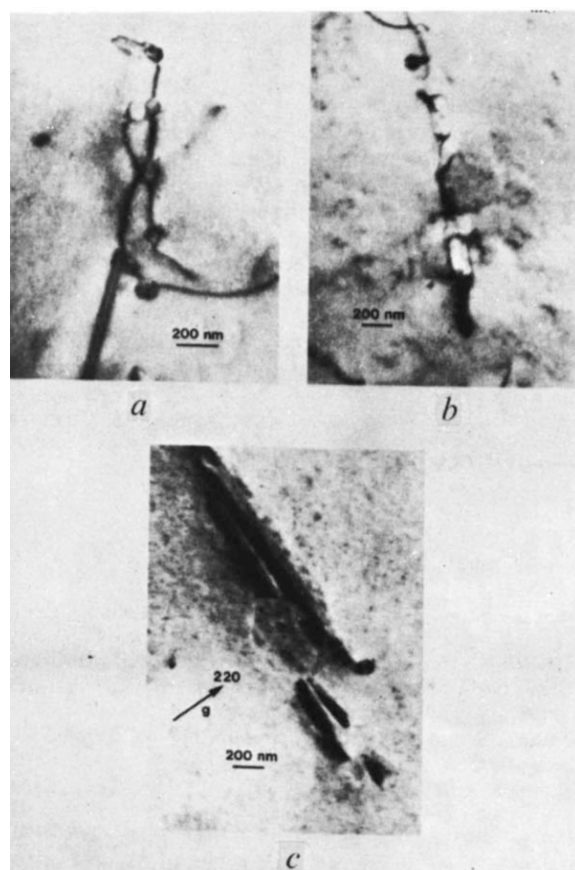


Fig. 1 *a*, Spherical inclusions preferentially nucleated along dislocation lines in KCl additively coloured with potassium to 10^{25} F m^{-3} and annealed for 1 h at 673 K to produce a colloid absorption band. *b*, Cylindrical inclusions along a dislocation line in KI additively coloured with potassium to $8 \times 10^{23} \text{ F m}^{-3}$ and annealed for 4 h at 433 K. *c*, Faceted inclusion in KI additively coloured with potassium, showing weak Moiré fringes normal to the diffraction vector *g*.

Transmission electron microscopy is a sufficiently direct technique but attempts⁵ to observe existing lattice defects in alkali halide crystals using this technique³³ have always been hampered by the catastrophic radiation damage induced by the investigating electron beam. New techniques^{34,35} involving electron microscopy at liquid helium temperature can circumvent this problem and allow almost routine electron microscopy of existing lattice defects; these methods have been applied recently to studies of dislocations in deformed crystals³⁶ and interstitial stabilisation in irradiated crystals³⁷. We have here applied them to the first direct electron microscopical observation of what we believe are alkali metal colloids in additively coloured alkali halide crystals.

Potassium chloride crystals (Harshaw Chemical Company) were additively coloured with potassium vapour, using van

Doorn's method³⁸, to produce an F-centre density (equivalent to a stoichiometric excess of potassium atoms) between 2×10^{24} and 10^{25} F m^{-3} . The crystals were sectioned parallel to $\{100\}$ and $\{110\}$, annealed in dry nitrogen or vacuum for 1 h at 673 K and either rapidly quenched or furnace-cooled. Optical absorption measurements indicated a large absorption band peaking between 750 and 850 nm, corresponding to average colloid diameters in the range 90–120 nm on the Mie theory. Potassium iodide crystals (Quartz et Silice) were similarly coloured to an F-centre density of $8 \times 10^{23} \text{ F m}^{-3}$, sectioned and annealed for 4 h at 433 K. Optical absorption measurements indicated a broad colloid absorption band peaking between 700 and 900 nm. Thin foils were prepared from annealed material using a precision chemical polishing method³⁹ and examined at liquid helium temperature^{34,35} in a 100-kV electron microscope. Approximately 15 s of observation time was available for image recording in KCl under an incident electron beam intensity of 18 A m^{-2} before aggregation of interstitial radiation defects impeded observation^{35,37}; approximately 45 s was available in KI at 45 A m^{-2} .

We observed images from inclusions (Fig. 1*a-c*) which we ascribe to potassium metal colloids. The observed inclusion dimensions vary between 50 nm and > 400 nm, those of the latter size being of the order of the foil thickness. When small, these inclusions are approximately spherical; larger inclusions are often faceted (Fig. 1*c*). They were observed by structure factor contrast^{40,41} appropriate to coherent precipitates with different extinction distances ξ_g from the matrix ($\xi_{220}(\text{KCl}) = 87 \text{ nm}$; $\xi_{220}(\text{KI}) = 70 \text{ nm}$; $\xi_{220}(\text{K, f.c.c.}) = 170 \text{ nm}$). Absorption contrast was also observed. The distribution of inclusions was found to be microscopically inhomogeneous, the average density being of the order 10^{17} m^{-3} . Independent measurements using a surface replication technique²⁶ confirm this size distribution, inhomogeneity and morphology. The number of potassium atoms which could be stabilised in aggregates of this size and distribution adequately accounts for the measured loss of F centres during anneal.

Absence of any additional diffraction features in selected area diffraction patterns from regions containing these inclusions suggests that potassium atoms are retained in their original face-centred cubic (f.c.c.) positions within the alkali halide matrix, with a lattice parameter not too different from the matrix ($a_0(\text{KCl}) = 0.629 \text{ nm}$; $a_0(\text{KI}) = 0.707 \text{ nm}$; $a_0(\text{K, f.c.c.}) = 0.655 \text{ nm}$; $a_0(\text{K, b.c.c.}) = 0.535 \text{ nm}$ at 300 K). When the inclusions were sufficiently large, weak Moiré fringes were observed (Fig. 1*c*) similar to those observed⁴² for misfitting coherent precipitates. The fringe spacing is not well defined, but nevertheless corresponds to a coherency strain⁴³ magnitude of the order 0.5% in both KCl:K and KI:K. The actual differences in alkali metal and alkali halide lattice parameters correspond to expected coherency strains of 0.31% for KCl:K (matrix in compression) and -0.76% for KI:K at 300 K (matrix in tension), using for the bulk modulus of f.c.c. potassium the value (4 GN m^{-2}) available for body-centred cubic (b.c.c.) potassium metal. The smallness of the strain values arises from the large compressibility (small bulk modulus) of the alkali metal lattice compared with the stiffness of the alkali halide matrix. Little matrix strain-field contrast^{44,45} is observed surrounding the larger inclusions as expected from the strain field visibility limits established by Ashby and Brown⁴⁴.

In crystals which were rapidly quenched from the annealing temperature, inclusions were often surrounded by dislocation tangles. When care was taken to cool crystals slowly from the annealing temperature to preserve the existing dislocation structure, inclusions were observed to have preferentially nucleated along existing dislocation lines (Fig. 1*a* and *b*). In such cases, they were more uniform in size and were regularly spaced along the dislocation lines; their shape was distorted in the direction of the dislocation line. This observation confirms the special role of dislocations in sensitising the nucleation of colloids. We have so far also made observations on similar inclusions in the systems KBr:K and NaCl:Na.

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The aerodynamic diameter of branched chain-like aggregates

AGGREGATES of a large number of submicron primary particles are produced by industry and automobiles. Such aggregates are also used in laboratory studies, so the fluid drag on these aggregates is of considerable interest.

Vomela and Whitby¹ have studied the fluid drag on chain-like aggregates, produced by an exploding wire as well as by hydrocarbon flames. Their data show a Stoke's diameter (diameter of a sphere having the same bulk density and settling rate as the aggregate) larger than the volume equivalent diameter (diameter of a sphere having the same volume as the aggregate) of an aggregate, which obviously cannot be correct⁴.

We deal here with aggregates of submicron iron oxide particles, produced by an exploding wire in an aerosol tank. The aerosol is labelled with ⁵⁹Fe by pre-irradiation of the wire with neutrons. The primary particle size distribution can be varied by varying the specific explosion energy.

Some time after the explosion, when aggregates are formed due to coagulation, the aerosol is sampled and analysed with a centrifugal spectrometer according to Stöber and Flachsbarth², which deposits the aerosol particles on a strip according to their aerodynamic diameter (diameter of a unit density sphere having the same settling rate as the aerosol particle), which is characteristic for the fluid drag.

The relationship between the mass and the aerodynamic diameter of the aggregates can be obtained from measurements of the radioactivity and particle number (obtained by counting from optical and for small aggregates from electron microscope pictures) along the deposition strip. The primary particle size distribution is determined using electron microscopy, with negatively stained catalase crystals³ as an internal length standard. It has been shown that the primary particle size distribution can be approximated by a log normal size distribution (characteristic features: the geometric mean diameter d_{1g} and the geometric standard deviation σ_g). The number n of primary particles of the aggregate can be calculated from the equation:

$$m = (\pi/6)\rho n d_{1g}^3 \exp(4.5 \ln^2 \sigma_g) \quad (1)$$

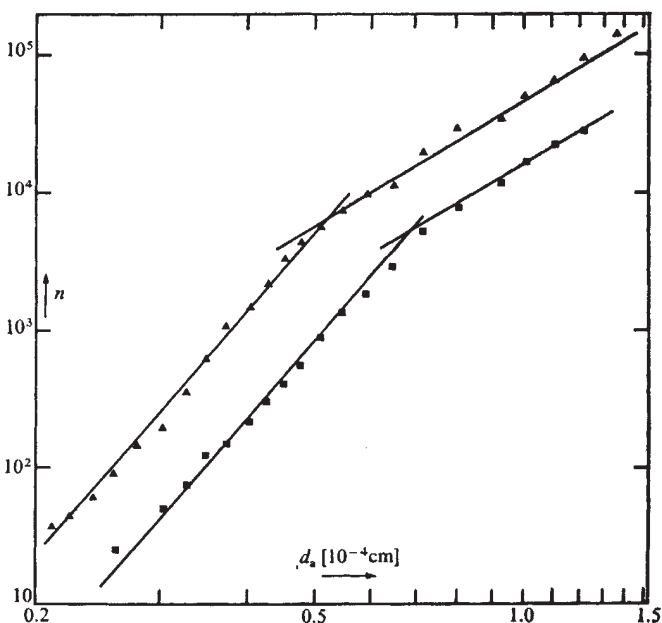


Fig. 1 The relationship between the number (n) of primary particles and the aerodynamic diameter (d_a) of branched chain-like aggregates of submicron iron oxide particles. $\blacktriangle$, $d_{1g} = 0.027 \mu\text{m}$, $\sigma_g = 1.8$; $\blacksquare$, $d_{1g} = 0.041 \mu\text{m}$, $\sigma_g = 1.8$.

with: m = mass of the aggregate and ρ = density of the aerosol material = 5.24 g cm^{-3} for the particles studied by us (aerosol material is Fe_2O_3 as could be determined by means of X-ray diffraction).

With the aid of equation (1) the relationship between the number of primary particles and the aerodynamic d_a of the aggregates can be obtained from the measurements of the aerosol mass and number distributions on the deposition strip in the aerosol centrifuge.

Figure 1 shows those experimental results already obtained for two different primary particle size distributions. For $n \leq 5 \times 10^3$, d_a is proportional to $n^{1/6}$, whereas for $n \leq 5 \times 10^3$ d_a is proportional to $n^{1/3}$.

Figure 2 shows that the configuration of aggregates with less than about 5×10^3 primary particles is linear in contrast to

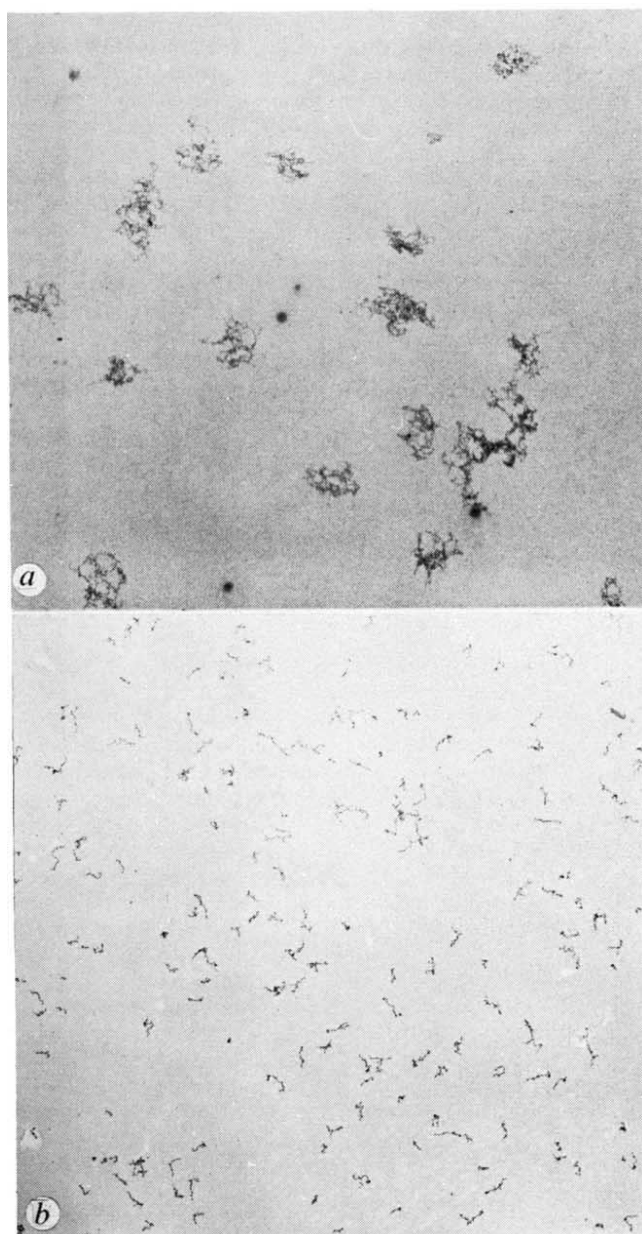


Fig. 2 Electron microscope pictures of branched chain-like aggregates of submicron iron oxide particles ($d_{1g} = 0.027$, $\sigma_g = 1.8$). a, $d_a = 0.925 \mu\text{m}$, $n = 3.10^4 (\times 165)$; b, $d_a = 0.259 \mu\text{m}$, $n = 100 (\times 700)$.

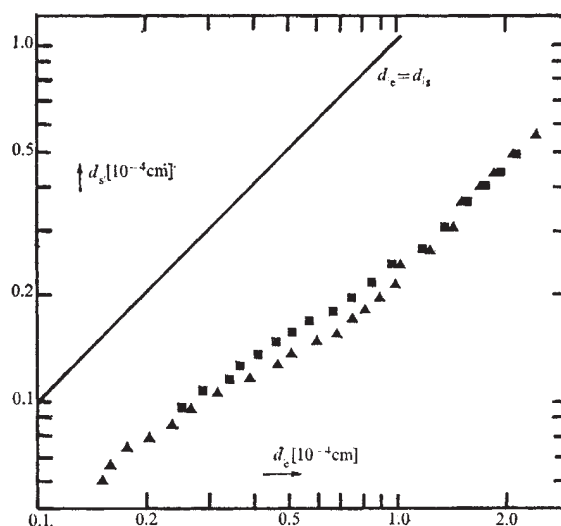


Fig. 3 Relationship between the volume equivalent diameter (d_e) and the Stoke's diameter (d_s) of branched chain-like aggregates of submicron iron oxide particles. $\blacktriangle$, $d_{1g} = 0.027 \mu\text{m}$, $\sigma_g = 1.8$; $\blacksquare$, $d_{1g} = 0.041 \mu\text{m}$, $\sigma_g = 1.8$.

aggregates with more than about 5×10^3 primary particles, which are irregular three-dimensional networks.

In accordance with Stöber⁴, the following relationship can be derived for the linear aggregates of polydisperse primary particles

$$d_a = kn^{1/6} \sqrt{(\rho/\rho_0)d_{1g} \exp(2\ln^2\sigma_g)} \quad (2)$$

with k = proportionality constant, $\rho_0 = 1 \text{ g cm}^{-3}$.

The values of k resulting from our experiments ($k = 0.92$ and 0.87 respectively) are somewhat smaller than Stöber's result ($k = 1.077$) for linear aggregates (up to eight primary particles) of monodisperse polystyrene spheres.

The relationship between the number of primary particles and the aerodynamic diameter of the aggregates with more than 5×10^3 primary iron oxide particles, has been found to be

$$d_a = fn^{1/3} \sqrt{(\rho/\rho_0)d_{1g} \exp(1.5\ln^2\sigma_g)} \quad (3)$$

with: $f = 0.903$ for cluster aggregates as results from Stöber's⁴ study; $f = 0.250$ for branched chain-like aggregates with more than about 5×10^3 primary particles, as results from this study.

The difference between the values of f found can be ascribed to the totally different packing densities of the aggregates studied by Stöber (cluster aggregates of monodisperse polystyrene spheres) and ourselves (fluffy aggregates of submicron iron oxide particles).

The relationship between the Stoke's diameter, which can be calculated^{4,5} from d_a and the volume equivalent diameter of the aggregates, is shown in Fig. 3. In contrast to the data of Vomela and Whitby¹ we found Stoke's diameters of the branched chain-like aggregates smaller than their volume equivalent diameters, which is in agreement with theoretical considerations.

The present results are of great importance, for example, in describing coagulation of solid aerosol particles. Until now the approximation of the droplet model was used in which the particles are assumed to be droplets which unite to form one new droplet⁶. In fact, this implies that the Stoke's diameter of chain-like aggregates equals the volume equivalent diameter. Obviously, this is incorrect.

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Decline of PCB concentrations in North Atlantic surface water

POLYCHLOROBIPHENYL (PCB) concentrations in North Atlantic surface waters have declined fortyfold since 1972, following the cessation of certain industrial uses of those compounds. Industrial sales of PCB for use in products which would allow leakage to the environment, for example in plastics, inks and paints were stopped in the United States and Sweden during 1970-71¹; and in 1972-73 other European countries and Japan initiated a similar action². In 1971 and 1972 average surface concentrations of PCB in the open North Atlantic Ocean were about 30 ng l⁻¹ (ref. 3 and C. E. Olney and J. J. Quinn, unpublished). By April of 1973 the concentration in the Sargasso Sea had been reduced to 1 ng l⁻¹ (ref. 4); five months later we found that the decline had levelled at 0.8 ng l⁻¹, a concentration which was still present in February 1974 in the north-west Atlantic. The accumulated data are presented in Table 1. These observations revealing a reduction in surface water concentrations of PCB compare well with our February 1974 measurements of a tenfold decrease of PCB in the atmosphere over the north-west Atlantic since 1973 (ref. 5), and a fivefold decrease

since 1972 in the PCB content of mixed plankton collected along 09°N latitude in September of 1973 (ref. 6).

The data require that about 2 × 10⁴ tons of PCB were lost from the upper 200 m in less than one year³. To explain this loss, four processes must be seriously considered: (1) apparent dilution of dissolved PCB by vertical and horizontal advection and diffusion; (2) association of PCB with sedimenting particles; (3) evaporative codistillation into the atmosphere and (4) biological and chemical degradation.

The information obtained from the monitoring of bomb fallout radionuclides is relevant to the present problem because like PCB, they are delivered to the ocean from the atmosphere, can exist in dissolved and particulate phases, and experienced a similar decreased input after the nuclear test ban agreements of

Table 2 PCB concentrations in North Atlantic subsurface waters*, 1973

Date of collection and analyses†	Position Latitude	Longitude	Depth (m)	PCB (ng l ⁻¹)
19/9/73	09° 00' N	40° 00' W	10	4.3
			100	2.0
			200	1.4
			400	1.1
			500	1.3
			700	1.5
			1,000	1.0
			2,000	2.1
2/10/73	32° 25' N	70° 20' W	3,000	1.0
			10	0.6
			100	0.8
			300	0.9
			600	0.5
			900	1.9
			5,100	0.4

* Reproducibility at the 0.5 ng l⁻¹ level was ± 40%. The blank was produced by recycling 40 l of XAD-2 extracted seawater through the entire procedure and averaged 0.3 ng l⁻¹.

† Method C was used.

1963. For example, strontium-90 which is mainly in the dissolved state in seawater, required more than five years after the test ban treaty to decline to one-half the surface water concentrations of 1963 (ref. 7). Thus, depletion of dissolved PCB from the upper ocean by advection and diffusion seems too slow to explain the observed rate. The rate of PCB removal from the surface waters more closely resembles the behaviour of the plutonium nuclides ²³⁹-²⁴⁰Pu which are believed to be associated with particulates⁸. The vertical distribution of plutonium isotopes in the Atlantic was best explained as being associated with particles falling at rates of 70-392 m yr⁻¹. The well known affinity of PCB for solid surfaces suggests that adsorption on falling particles could be a major transfer mechanism. In fact, if 2 × 10⁴ tons or less of PCB has been transferred to the deep ocean each year (1970 was the peak production year) for the past 10-15 yr and was dispersed throughout the entire water column, the concentrations to 3,500 m would still be less than 2 ng l⁻¹. Two 1973 profiles of PCB in the water column are presented in Table 2. The profile at 40° 00'W was typical of eight other stations in the North African and Cape Verde Basins; in contrast, the station at 70° 20'W revealing very low PCB levels at the surface, was similar to nine other stations in the North American Basin the same year.

We are, at present, unable to ascertain the relative importance of evaporative codistillation of PCB from the surface water or of biogeochemical degradation. Either process could explain the lower surface water concentrations in the North American Basin. There is, as yet, no evidence of biological metabolism of PCB in the marine environment.

We note the rapid response of the open-ocean mixed layer to the reduction of industrial discharges of PCB. Efforts to

Table 1 PCB concentrations in North Atlantic surface waters* 1971-74

Date	Mean PCB† (ng l ⁻¹)	Sampling region	No. of samples	Analytical‡ method	Ref.
8/71	25	Iceland to Nova Scotia	8	A	§
7/72	41	Newfoundland to Portugal	8	B	3
8/72	36	Portugal to Norwegian Sea	11	C	3
9/72	30	Woods Hole to Bermuda	27	C	3
4/73	1	Sargasso Sea	8	A	4
9/73	2	Azores to 09°N, 40°W to Barbados	10	C	3
9/73	0.8	Sargasso Sea to New York Bight	9	C	3
2/74	0.8	New England Continental Shelf	6	C	3

* 0-30 m.

† Blank values for PCB in methods A, B and C were 0.9, 0.5 and 0.3 ng l⁻¹, respectively. Replicate analyses using methods B or C showed an average variation of ± 20% at the 1 ng l⁻¹ level. Quantitation methods are presented in refs 3, 5, 6.

‡ A, Extraction of 0.5-3.8 l with chloroform or dichloromethane; B, extraction of 20 l with 5% ether in hexane; C, passing 40-60 l through Amberlite XAD-2 resin followed by elution with boiling acetonitrile.

§ C. E. Olney and J. J. Quinn, unpublished information.

determine the mechanism of this efficient transfer process are in progress.

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Origin proposed for non-protein amino acids in meteorites

THE amino acids that have been identified in several meteorites^{1,2} can be divided into two classes: those present in proteins and those which are not. The presence of the non-protein amino acids, D- and L-β-aminoisobutyric acid and β-alanine, is described as evidence for the indigenous origin of meteorite amino acids and against terrestrial contamination¹. It is implied that these β-amino acids arise by prebiotic condensation of ammonia, methane, hydrogen, water and other simple primordial molecules³. The β-amino acids and other amino acids can arise from simple molecules by Fischer-Tropsch synthesis in the laboratory. Hydrogen, carbon dioxide and ammonia were heated to high temperatures in the presence of natural catalysts expected to be present in the solar nebula⁴.

In other experiments, thermal synthesis of amino acids occurred in a simulated primitive atmosphere of methane, ammonia and water, followed by acid hydrolysis of the products. β-Alanine was obtained in high yield, relative to other amino acids, and the straight-chain compound, β-amino-n-butyric acid⁵. These amino acids were postulated to arise respectively from hydrolysis of β-aminopropionitrile and the nitrile intermediate arising from addition of ammonia to crotononitrile. Evidence for this route was the identification of succinic acid and N-methyl-β-alanine which are likely hydrolysis products of nitrile intermediates. Production of β-aminoisobutyric acid was not reported in these experiments⁵.

β-Aminoisobutyric acid is a rare amino acid with a curious distribution in nature. It has been isolated only from human urine⁶, iris bulbs⁷, flatworms⁸ and edible mussels⁹. Man is probably the only mammalian species to excrete D-(−)β-aminoisobutyric acid¹⁰ though there is chromatographic evidence for its presence in rabbit urine¹¹. Metabolic experiments suggest that the pyrimidine base thymine, from nucleic acid catabolism, is the precursor of β-aminoisobutyric acid in the human¹²⁻¹⁴. Similarly, metabolic production of β-alanine in rat liver is from the pyrimidine uracil¹⁵.

By analogy with these known reaction sequences catalysed biologically, I suggest that heterocyclic compounds could also act as precursors for the β-amino acids found in meteorites. In this case, scission of the ring of heterocyclic compounds formed prebiotically would occur under the catalytic conditions of extremes of temperature and radiation thought to occur in space.

The purines, adenine and guanine, and a substance resembling uracil have been detected in the Orgueil meteorite¹⁶. Xanthine, thymine, uracil and derivatives have also been obtained experimentally by mimicking supposed prebiotic conditions⁴.

This chemical route to certain non-protein amino acids described above would presumably give racemic mixtures of both stereoisomers, for example, both D-, and L-β-aminoisobutyric acid, as found in meteorites¹. It would be interesting to know if thymine, dihydrothymine, uracil and related compounds, when heated under supposed prebiotic conditions, did give traces of the β-amino acids.

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Evidence for downwind flights by host-seeking mosquitoes

THE behaviour of flying insects which make use of airborne chemicals in their search for food, oviposition sites or sexual partners can be separated into two phases, the search flight bringing them into contact with the attractant plume and the approach flight leading them to its source^{1,2}. The second phase is characterised by upwind orientation in response to the appropriate chemical stimuli³⁻⁶. Mosquitoes are known to fly upwind as they approach a warm-blooded host⁷⁻⁹. For most insects relatively little is known of the pattern of the preceding search flight which Haskell² described as 'wandering', but where the wind is light it may be upwind¹. Experiments in West Africa have shown that the majority of mosquitoes entered flight traps from the downwind side, that is they appeared to have been flying upwind, regardless of the presence or absence of a host (ref. 10 and W. F. Snow, unpublished). Here we report experiments that unexpectedly provide evidence for the opposite type of behaviour.

If the search flight is in a generally upwind direction, it should be possible to divert mosquitoes round a host by setting up a barrier on the downwind side and so provide a measure of protection from their attacks. The barrier would need to be sufficiently far away for the mosquitoes to encounter it before they detected the presence of the host, so that directed responses towards the host would not be elicited. This distance was estimated by Gillies and Wilkes¹¹ to be less than 18 m for a host the size of a man. A host stationed at this distance upwind

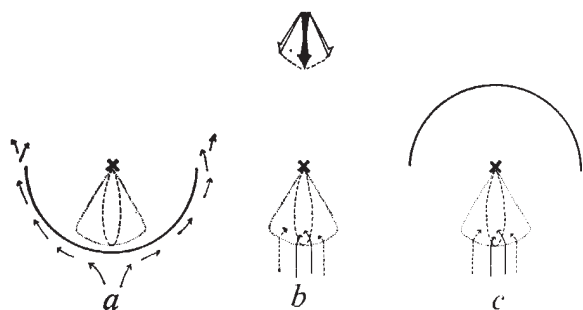


Fig. 1 The effect of barriers on mosquitoes flying upwind towards a host. Crosses represent hosts protected by, *a*, semicircular barriers on the downwind side; *c*, upwind side; and, *b*, unprotected. The thick arrow represents wind direction, and the fine arrows the flight paths of mosquitoes. The plume of attractant from the host is shown as an ellipse, swinging in a narrow arc in a variable wind.

of the barrier should, therefore, receive fewer bites than a bait in the open or one protected on the upwind side (Fig. 1).

A bait stationed downwind of the barrier (Fig. 1 *c*) might well have been within its wind shadow which could have affected the density of insects on the leeward side regardless of their direction of flight^{1,2}. So we decided to compare the attack rate on a host upwind of a barrier with that on an unprotected bait at the points marked X in Fig. 1 *a* and *b*. A semicircular fence of plastic mosquito netting was constructed with the opening perpendicular to the prevailing wind. The fence was 2.9 m high, 57 m long and formed part of a circle of radius 18.3 m. It was set up in an extensive area of cleared farmland near Sapu, The Gambia, 0.5–1 km from the nearest village. Two human 'baits' sat 36 m apart at points *a* and *b* (Fig. 1) and caught the mosquitoes in tubes as they settled to bite, changing places at halftime. Wind speed was measured at a height of 1 m and wind direction was recorded at 1–2 min intervals. Weather conditions were favourable on eight nights in July 1973, for a total catching time of 6.5 h. On these nights the wind was within 45° of the prevailing direction for 96% of observations. Mean wind speed was 2 m s⁻¹. The fence was highly permeable, air flow at a point 3 m downwind from it being reduced by a factor of 0.62 at ambient wind speeds.

In a second series of experiments, the half-circle of fence was converted into a complete circle of the same height. Paired catches by human bait were done in the same way as before, except that the control bait was 70 m from the nearest part of the fence. Mean wind speed in the open was 0.9 m s⁻¹.

The results with the half-circle fence showed that the mean nightly ratio of mosquitoes caught upwind of the fence to those in the open was 0.97. Thus virtually identical numbers were caught by the 'protected' and by the exposed bait (total catch 814 mosquitoes, 84% being *Mansonia (Mansonioides)* spp.). On the other hand, the results of 12 paired catches inside and outside the complete circular fence showed that the mean nightly ratio of catches inside to catches in the open was 0.69.

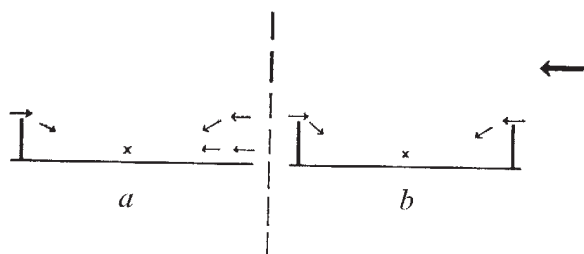


Fig. 2 Section through, *a*, semicircular fence and, *b*, circular fence to show possible approach paths of mosquitoes (fine arrows) to the host (X). Wind direction is indicated by the thick arrow on the right.

The difference for catches of *Mansonia* spp., which totalled 2,048 mosquitoes, was highly significant ($P < 0.01$). This shows that the circular fence reduced the numbers reaching the bait by something like 30%.

With the half-circle fence mosquitoes could have reached the bait by flying downwind at any level or upwind only over the top of the fence (Fig. 2*a*). The circular fence would have excluded low-level fliers and only permitted the approach of high-flying insects (Fig. 2*b*). If the mosquitoes that reached the bait in the first experiment had mainly approached by flying over the fence, the exclusion of low-flying insects in the second experiment would not have had any effect on the catch. As, on the contrary, there was a marked reduction in this catch, we conclude that a significant number of mosquitoes reached the vicinity of the bait in the first experiment by flying at a low level downwind. Their search flight before the detection of host stimuli must therefore have been in a generally downwind direction and low enough for them to encounter the odour plume from a ground level host. Thereupon they presumably turned and tracked back upwind toward the host. This would make for a much more efficient host-seeking strategy since, for a given output of energy, the insect would be able to cover a much larger area than if it were restricted to upwind movements.

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Diffuse competition in Lepidoptera

THE effects of interspecific competitive encounters have traditionally been viewed in terms of species replacement, but recent workers^{1,2} have recognised the importance of weak or diffuse competition, which leads only to reduced niche volume. Evidence of such niche restriction has been obtained from the study of bird communities, where it has been shown that species often expand niche dimensions in areas with few species^{2,3}. For example, one-third as many bird species are present on the island of Puerco as on the nearby Panamanian mainland and the insular birds forage in a wider range of habitat types than conspecific mainland birds⁴. Unfortunately this rather direct method of relating changes in niche width to changes in competitive background is often difficult to apply but the importance of competition can be evaluated in other ways. It is generally conceded that if competition occurs at all, it will be strongest among phenotypically similar species⁵.

More explicitly, the intensity of competition which a species faces should be inversely related to its phenotypic isolation, where such isolation is a complex function of the phenotypic distance between a species and coexisting species which share limiting factors.

The relationship between intensity of competition and species abundance is not likely to be a simple one. Much depends on the limiting factors involved in the competition. The abundance of resource-limited species is, on the short run at least, related to the abundance of the resource. The data from Puerco⁴ do, however, indicate a flexibility in resource abundance, depending on the utiliser's definition of the resource. The potential overlap of such definitions will clearly lead to competition. In cases where resource limitation is unimportant, the effects of competition on abundance could be equally important, but not necessarily any more clear-cut; few limiting factors are totally nonspecific, yet, equally few are totally specific.

It is difficult to make a general case for the resource limitation of herbivorous insect populations. Certainly, as Hairston, Smith and Slobodkin⁶ point out, insects rarely strip their food plants of foliage. In non-resource limited species the possibility of competition arises when several species share mortality factors⁷ such as parasites, predators or disease, for a group of species will then tend to be regulated as a whole rather than as individual units.

We decided to analyse patterns of abundance in lepidopterans because large numbers of species are present in a local fauna. Moreover, the families to which these species belong are represented by differing numbers of species. Species which belong to small families will be more phenotypically isolated from the lepidopteran community as a whole than species belonging to families represented by many species and the present study demonstrates that species in these small families tend to have higher population densities.

As it is difficult to sample the abundance of an entire lepidopteran community in any meaningful way, this study has been restricted to an analysis of the abundance of night flying moths. To reduce the size variation of the species considered and to avoid taxonomic difficulties, Microlepidoptera were

Approximately the same number of species per family were obtained at site 2 although only 307 Noctuidae and 140 Geometridae were collected.

Large differences in family abundances as measured by the arithmetic mean number of individuals per species were observed (Table 1). The Noctuidae were represented at both sites by the lowest average number of individuals per species, while the Geometridae were next rarest. Species belonging to the three intermediate-sized families were represented on average by twice as many individuals as the noctuid species, while the species belonging to the smaller families were still more abundant. Abundance values based on arithmetic means have the disadvantage that a single extremely common species can influence strongly the result obtained. To rule out the importance of this effect in our results, the abundance of each species was

Table 2 Family differences in species abundance, based on mean $\log_2$ abundance

	Site 1		Site 2	
	No. of species	Mean abundance	No. of species	Mean abundance
Noctuidae	401	4.12	307	3.00
Geometridae	168	4.56	140	3.51
Notodontidae	43	5.86	43	4.68
Arctiidae	34	5.65	28	4.46
Sphingidae	22	5.35	21	4.21
Other families	32	6.16	32	4.53

converted to a $\log_2$ scale and placed in a discrete abundance class or octave⁹; family abundance was based on the mean $\log_2$ abundance of the species in the family. Half of the species represented by just a single specimen were thus omitted from the calculations, as they belong to an incomplete frequency class^{9,10}. This omission leads to anomalously high mean abundance values in families with many species represented by single individuals, an effect which reduced the abundance differences between families in the present data. In spite of this, the same trend in family abundance was observed (Table 2). The Geometridae were consistently more abundant than the Noctuidae, the Notodontidae, Sphingidae and Arctiidae were roughly 1.5 times as abundant as the Noctuidae, while the other families averaged the highest abundance of all.

Family differences in light trap abundance could result from differences in phototropicity. It is difficult to suggest, however, why an orderly relationship should exist between the number of species in a family and the phototropicity of the species included in it. On the other hand, a relationship between species number and 'real world' abundance is expected if competitive interactions are important. Additional evidence of competition among lepidopterans has been obtained by analysing the effects of environmental disturbance on species abundance and by studying the relationship between the number of species in a habitat and species abundance (P.D.N.H., P.S.W., and R.H., unpublished). It seems justified to conclude that the family differences in light trap abundance observed in the present study reflect real world differences in abundance and that these differences are due to competitive interactions among species. The mechanisms by which competition is mediated are not clear, although shared mortality factors must be important. Certainly, competition of this sort could be intensified at the family level. A bird searching for the stick-like larva of one geometrid species might well be more likely to discover the larva of another geometrid species than the bizarrely-shaped leaf-mimicking larva of a notodontid. Similarly it is known that many lepidopteran parasites are not species-specific but attack an array of related species¹¹.

Our results conflict with the view that insect populations¹²⁻¹⁴ are regulated largely by density-independent factors, a view which has been based on the results of case history studies of single, necessarily abundant, species. As our study suggests that the common species in a community are often phenotypically isolated from competitors, it is not surprising that

Table 1 Family differences in species abundance

	Site 1*		Site 2†	
	No. of species	No. of individuals/species	No. of species	No. of individuals/species
Noctuidae	401	68.61	307	21.36
Geometridae	168	91.51	140	27.79
Notodontidae	43	156.02	43	72.42
Arctiidae	34	201.35	28	50.54
Sphingidae	22	130.91	21	42.90
Ten small families	32	223.84	32	70.84

One 'sample' is a collection from one light over one season.

*Perth Road, Frontenac Co., Ontario (1970-1971); four samples, total no. of individuals = 66,484.

†Chaffey's Locks, Leeds Co., Ontario (1969-1970); four samples, total no. of individuals = 18,146.

excluded from study. Collections were carried out at two sites approximately 10 miles apart. Site 1 was situated in a large area of undisturbed deciduous forest, while site 2 was situated in an area which had been subject to limited clearing. Moths were collected with a 20 W ultraviolet light set against a white cloth background. Trapping periods have been listed elsewhere⁸, but generally extended from May until mid September.

Fifteen families of moths were represented in the collections. At site 1, 401 species of Noctuidae, 168 species of Geometridae, 43 species of Notodontidae, 34 species of Arctiidae and 22 species of Sphingidae were collected. Ten additional families (Citheroniidae, Drepanidae, Epipleidae, Euchromiidae, Lasiocampidae, Lymantriidae, Nolidae, Saturniidae, Thyatiridae and Zanolidae) were represented by from one to seven species.

their study has resulted in a misleading impression of the importance of species interactions.

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In vitro phagocytosis of bacteria by insect blood cells

PHAGOCYTOSIS is an important component of vertebrate cellular immunity and involves several stages which have been described in detail previously^{1,2}. In contrast, little is known about this process in insects even though recent work^{3,4}, on intracellular killing of bacteria indicates that phagocytosis probably plays an important role in the defence reactions of insects. In vertebrates, the development of *in vitro* techniques has greatly facilitated studies on phagocytosis, but in insects such techniques are not generally available for studying the blood cells (haemocytes) in strictly controlled conditions. Here we describe for the first time the stages in the phagocytosis of

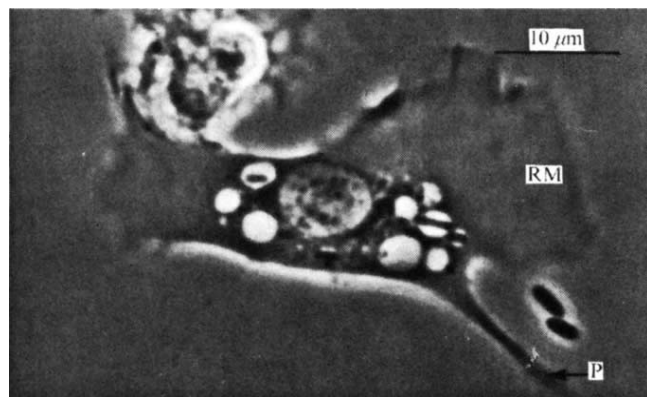


Fig. 1 Plasmatocyte with intracellular *E. coli* surrounded by large phagocytic vacuoles. Cell shows characteristic cell spreading, ruffled membranes (RM) and protoplasmic extension (P). Haemocyte:bacteria ratio 1:70; incubation time 30 min; phase-contrast optics.

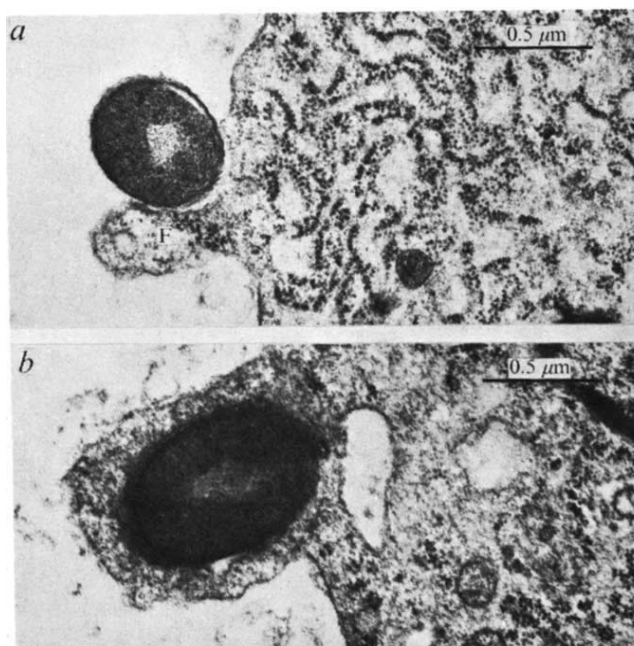


Fig. 2 Stages in attachment and ingestion of *E. coli*. *a*, Attachment of *E. coli* to plasmatocyte cell membrane. Note small filopodial extension (F). *b*, Plasmatocyte with ingested bacterium projecting from cell surface. Cells fixed in 2:1 (v/v), ice-cold 1% osmium tetroxide and 2.5% glutaraldehyde, postfixed in 0.25% uranyl acetate⁵. Haemocyte:bacteria ratio 1:80; incubation time 45 min.

bacteria by insect haemocytes, using a newly developed culture technique.

Final instar larvae of *Galleria mellonella* (120–180 mg), were bled by severing a proleg and collecting the haemolymph into Grace's insect medium (Grand Island Biological Company) to give a final haemocyte concentration of $2-3 \times 10^5 \text{ ml}^{-1}$. All procedures were carried out in a nitrogen atmosphere to delay the toxic effects of haemolymph melanisation. *Escherichia coli* K 12 (NCTC 10538), were used as test particles in bacteria:haemocyte ratios of 20–100:1. Droplets (0.2 ml), containing haemocytes and bacteria were suspended in silicone oil in multi-dish trays (6 × 3.5 cm diameter wells; Flow Laboratories) and incubated at 26°C on an orbital shaker (45 r.p.m.) for 10–90 min. For light microscopy, the haemocytes were allowed to attach to glass coverslips after incubation, and the resulting monolayer examined unfixed.

Using previously defined criteria⁵, five cell types were identified in the haemolymph of *G. mellonella*; prohaemocytes, plasmatocytes, granular cells, spherule cells and oenocytoids but only the plasmatocytes and granular cells phagocytosed the bacteria. The plasmatocytes were easily identified by their extensive spreading on the coverslips to reveal intracellular details (Fig. 1), and had a much greater phagocytic ability than the granular cells, many of which underwent progressive degeneration *in vitro*.

The electron micrographs show that the characteristic attachment and ingestion phases of the phagocytic process in vertebrates², also occurred in *G. mellonella* haemocytes. Initially many of the bacteria became closely attached to the haemocyte surface (Figs 2*a* and 3*a*), and the cell reacted by forming fine pseudopods (filopodia), which extended over the bacterial surface (Fig. 2*a*). The tips of the filopodia eventually fused with each other, or with the haemocyte surface to enclose the bacteria completely in pockets of cytoplasm (Fig. 2*b*). In this type of ingestion no well defined phagocytic vacuoles were formed and the bacteria frequently appeared, in low power electron micrographs, to lie directly in the cytoplasm (Fig. 3*a*). This 'close contact' process explains previous light microscopy reports of engulfed particles lying in the haemocyte cytoplasm



Fig. 3 Intracellular bacteria. *a*, Plasmatocyte with three ingested *E. coli* (arrows). Note lack of phagocytic vacuoles, and uptake of cell debris (CD) by invagination of cell membrane. *b*, Bacterium within well defined phagocytic vacuole (arrow). Fixation and incubation as in Fig. 2.

without vacuole formation⁷. Sometimes however, ingestion occurred by the formation of phagocytic vacuoles (Fig. 3*b*) similar to those described previously in infected haemocytes⁸. In the light microscope, these vacuoles often appeared enlarged, probably as a result of the high degree of cell spreading in the monolayers (Fig. 1).

These results confirm previous descriptions of particulate ingestion by insect phagocytes⁸⁻¹⁰ and show that bacterial phagocytosis can occur by pseudopod formation alone. The uptake of larger particles, however, such as cell debris is probably accomplished by invagination of the cell membrane (Fig. 3*a*) to form intracellular vacuoles.

Finally, although lysosomes are known to occur in insect haemocytes^{11,12}, such bodies were not apparent in *G. mellonella* plasmatocytes. If phagocytosed bacteria are killed and degraded in the haemocytes, then the origin of the enzymes involved is unknown. We are at present utilising this *in vitro* technique to investigate this problem, together with other aspects of insect cellular defence reactions.

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Giemsa banding of metaphase chromosomes in triatomine bugs

CONSIDERABLE progress has been made in developing the formal genetics and cytogenetics of several insect vectors of disease^{1,2}, notably with mosquitoes, houseflies and tsetse flies in which the polytene chromosomes provide suitable material for detailed analysis of chromosome morphology. The triatomine bugs (Hemiptera, Reduviidae) are medically important as vectors of Chagas' disease in the Americas, yet cytogenetic information on these insects is meagre³⁻⁸. These bugs present the same problems which, until recently, limited developments in mammalian cytology in that they possess a large number (typically $2n = 22$) of small, almost indistinguishable chromosomes⁷. Further, since their chromosomes are also holokinetid⁹ (that is with non-localised centromeres) they do not show any primary constrictions and it is correspondingly difficult to recognise arms of a chromosome which are readily seen in chromosomes of organisms possessing discrete centromeres. This difficulty with triatomine material has now been overcome by applying the Giemsa staining methods, which have been effectively developed by mammalian cytologists¹⁰⁻¹³, to embryonic cells of bugs with metaphase configurations. The technique I describe here makes possible the identification of individual chromosomes within the complements of different species of triatomine bug.

The procedure is as follows: 5-7-d-old embryos are dissected out from bug eggs, placed in hypotonic sodium citrate solution (1% for 8 min) and the cells dispersed in 0.25% trypsin in



Fig. 1 Metaphase neuroblast from a *Triatoma infestans* male embryo 6 d old (20 + XY).

0.02% versene. The suspension is washed in insect ringer, centrifuged and fixed in alcohol-acetic acid. Slides are prepared by air drying in the normal way¹⁴. Dry slides are kept for 1 week at 20° C before heating at 60° C for 1 h in 2 × SSC (0.3 M sodium chloride; 0.03 M trisodium citrate). After rinsing in absolute ethanol, preparations are stained in 2% Giemsa (Gurrs 'improved' R 66) at pH 6.8 for 30 min, dried and mounted. This ASG (acetic-saline-Giemsa) technique¹⁰ has proved to be a more reliable method for the production of G bands in bug chromosomes than other methods which have also proved effective with mammalian cells.

Figure 1 shows banding and differentiation of metaphase chromosomes in *Triatoma infestans*, and indicates the results obtainable with this technique. Similar preparations have been obtained for other *Triatoma* species and also *Rhodnius prolixus*. Preliminary analyses indicate that there are prospects for production of chromosome maps of triatomine species which could be of value in further investigations of chromosome markers and their application to problems in genetic characterisation and manipulation in the vectors of Chagas' disease.

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Forced association between higher plant and bacterial cells *in vitro*

SYMBIOTIC associations with microorganisms enable many plant species to utilise molecular nitrogen¹⁻³. *In vitro* systems have been developed to study some of these natural associations⁴⁻⁷, and here we have attempted to define an experimental system for investigating the possibility of extending the nitrogen-fixing symbiosis to additional crop species. We used tissue culture techniques to force an association between the free-living nitrogen-fixing bacterium, *Azotobacter vinelandii* and cells of a carrot, *Daucus carota* cv. Danver's Half Long. The resulting composite callus proliferates slowly on a defined synthetic medium lacking combined nitrogen. In these conditions carrot cells which have not formed an association with *Azotobacter* are unable to survive.

To form the *Azotobacter* plant cell association, carrot cell suspensions growing logarithmically in a liquid medium⁸

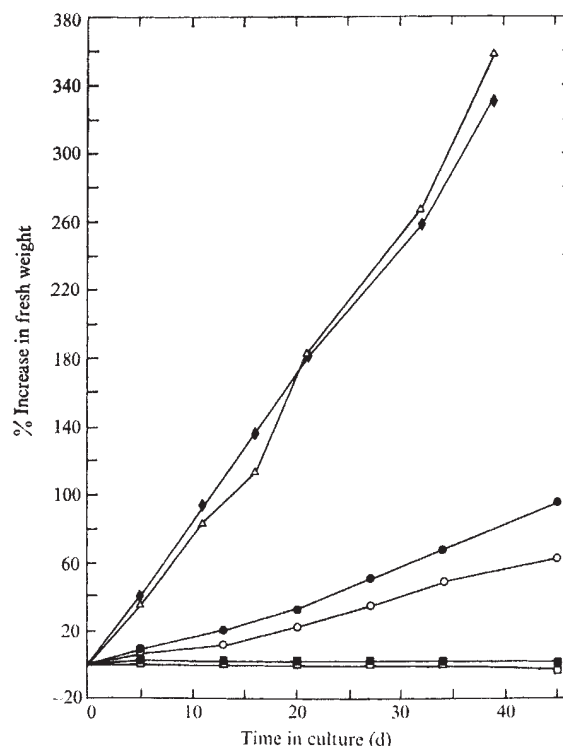


Fig. 1 Growth of *Azotobacter*-infected and uninfected control carrot tissues on media containing different levels of combined nitrogen. Carrot cells were grown on a Linsmaier and Skoog medium⁸, supplemented with 4% sucrose and containing 3 mg l⁻¹ indoleacetic acid and 3 mg l⁻¹ 6(γ,γ-dimethylallylamino)-purine (2iP). Media were solidified with 1% agar. NH₄NO₃ and KNO₃ were omitted from N-free medium. Medium which lacked NH₄NO₃ and contained 0.19 g l⁻¹ KNO₃ is referred to as low N medium. The adenine-auxotrophic strain of *Azotobacter vinelandii* (ATCC 25308) was grown on modified Burke's nitrogen-free medium (pH 7.8) comprising Na₂HPO₄ (0.189 g), KH₂PO₄ (0.011 g), MgSO₄·7H₂O (0.20 g), FeSO₄·7H₂O (6.0 mg), NaMoO₄·2H₂O (0.6 mg), CaSO₄·2H₂O (20 mg), SrCl₂·6H₂O (10 mg), NaCl (10 g), NaHCO₃ (50 mg), adenine HCl (20 mg) and sucrose (20 g) per l H₂O. All calluses were grown on low N medium for 3 weeks before being transferred to the medium indicated. Initial inoculum weight was approximately 50 mg. Control carrot tissue transferred to N-free (□), low N (■), or standard Linsmaier and Skoog (△) medium. *Azotobacter*-containing carrot tissue transferred to N-free (○), low N (●), or standard Linsmaier and Skoog (◆) medium.

containing standard nitrogen levels were inoculated with cells of an adenine-requiring strain of *A. vinelandii* to a final concentration of 10⁶ ml⁻¹ bacterial cells. After 12 d the mixed cultures were washed and suspended in N-free carrot medium. The cultures were incubated for an additional 2 weeks after which they were plated in N-free carrot medium solidified with 1% Noble agar. The plates were incubated at 23° C in a 16 h light/8 h dark cycle. The rare and slowly growing colonies were selected as they appeared 3-6 months after plating and were transferred to either N-free or low N medium. The rate of colony formation on N-free medium was approximately 10⁻⁴ to 10⁻⁵ of the total number of carrot cells plated. Control experiments in which carrot cultures were not inoculated with *Azotobacter* produced no colonies on N-free medium. Several independent lines of evidence demonstrate that the infected plant cultures depend upon *Azotobacter* for growth in the absence of combined nitrogen.

Carrot cells are unable to survive *in vitro* in the absence of combined nitrogen (Fig. 1). When 1.9 mM KNO₃ is supplied as the sole source of combined nitrogen carrot cells do not proliferate over a 4 month period. On these media calluses which have been recovered after inoculation with *Azotobacter* cells increase in fresh weight. On medium containing normal

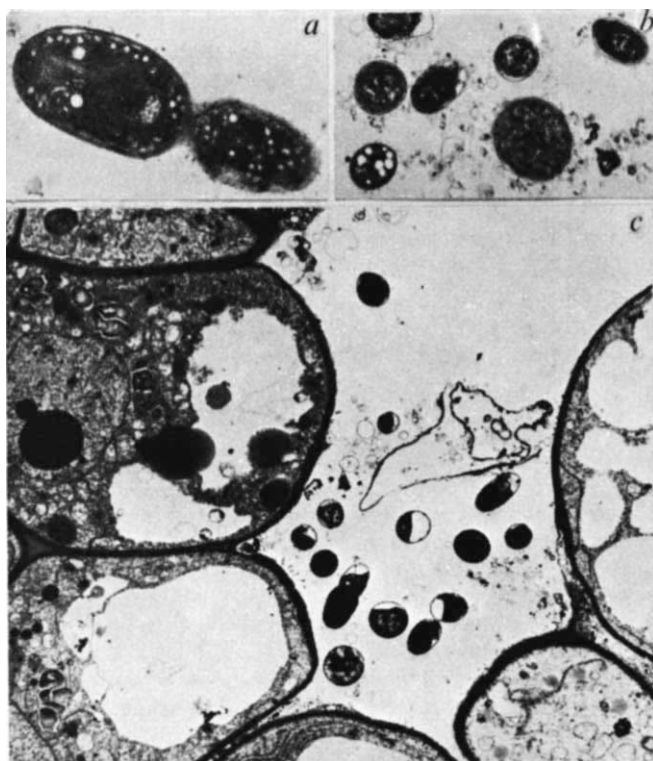


Fig. 2 Electron micrographs showing, *a*, the fine structure of the bacteria in the composite callus ($\times 13,036$); *b*, bacterial cells in the agar underlying the composite callus ($\times 2,743$); *c*, composite callus growing on low N medium ($\times 2,305$). Fixation of callus cultures growing on low N medium for electron microscopy was accomplished by flooding the agar plates with a 3% buffered solution of glutaraldehyde for 4 h, followed by a 6% solution of glutaraldehyde. After 4 d samples were excised from the callus and the underlying agar, washed, and postfixed in a 2% solution of OsO_4 overnight. The fixing solutions and wash were buffered with 0.05 M sodium cacodylate at pH 6.8. The samples were dehydrated in a graded series of acetone solutions at 0–2° C., and embedded in an epoxy resin. Thin sections were stained with uranyl and lead salts before viewing in the electron microscope.

levels of NH_4NO_3 and KNO_3 (20.6 mM and 18.8 mM, respectively) no difference in growth rates is observed between uninoculated calluses and inoculated calluses which are capable of growth on N-free medium. Inoculated calluses have been cultured for over 18 months and have maintained the ability to grow in the absence of combined nitrogen during that period.

The addition of penicillin G to the medium at a concentration which is known to kill *Azotobacter* cells ($50.0 \mu\text{g ml}^{-1}$) destroys the ability of the inoculated callus to grow on N-free medium. Growth of either inoculated or uninoculated calluses on medium containing normal levels of combined nitrogen is not inhibited by the addition of penicillin. These observations indicate that the ability of the inoculated callus to grow on N-free medium is dependent on the presence of functional bacterial cells in the callus mass.

Composite calluses capable of growth on N-free or low N medium and control calluses were suspended in T broth and in Burke's minimal and adenine-supplemented media. After several days turbidity developed only in the adenine-supplemented Burke's medium. This suspension was plated and identified as the original *Azotobacter* adenine auxotroph with which the calluses had been inoculated. None of these three media became turbid when inoculated with control carrot callus. The absence of growth in T Broth (which supports growth of a prototrophic *Azotobacter* strain, but not of the adenine-requiring strain) after a 3 week period indicates that the calluses contain no contaminating microorganisms.

Electron micrographs of inoculated carrot callus grown on low N medium clearly establish the presence of bacterial

Table 1 Acetylene-reduction activity

	Incubation period (h)	nmol C_2H_4 per ml gas $\pm$ s.d.
No tissue	10	0.22 ± 0.05
<i>Azotobacter</i>	1	1.46 ± 0.16
Carrot callus	10	0.26 ± 0.10
Carrot- <i>Azotobacter</i> callus	0	0.25 ± 0.11
Carrot- <i>Azotobacter</i> callus	10	0.71 ± 0.07

Approximately 50 mg fresh tissue was placed in 1 ml vials which were then injected with 0.10 ml acetylene. The *Azotobacter* controls contained approximately 8×10^7 cells in 0.10 ml modified Burke's medium. Reactions were stopped by the addition of 0.1 ml 0.1 N H_2SO_4 . The ethylene content of samples was determined by published gas chromatographic procedures¹¹. The composite callus does not evolve ethylene in the absence of acetylene. It is presumed that the level of ethylene found in the absence of tissue was present as a contaminant in the acetylene used in the assay.

cells in the intercellular regions of the tissue (Fig. 2c) and in the underlying agar (Fig. 2b). Live bacteria were not observed within the carrot cells. The fine structure of the bacteria growing with the callus cultures (Fig. 2a) was comparable in all essential details to *Azotobacter vinelandii*^{9,10}. No other organisms were found with the callus or in the agar.

Azotobacter-containing carrot tissue capable of growth on N-free medium evolves significantly more ethylene in the acetylene assay for nitrogenase than does an uninoculated control callus (Table 1).

The association between cells of carrot and *Azotobacter* has been accomplished by establishing conditions of mutual dependency in culture. Presumably, carrot cells receive reduced nitrogen from *Azotobacter* cells which, in turn, depend upon the carrot cells to satisfy their auxotrophic requirement for adenine. This association is not completely stable. Occasionally the callus segregates some cell masses which have lost the ability to grow on N-free medium while others retain this ability. Other calluses become overgrown by bacterial cells. Most of the composite calluses (>90%), however, have maintained a stable association over the 18 months they have been in culture.

An assessment of the usefulness of the carrot-*Azotobacter* association and of the magnitude of its effect must await regeneration of plants from the composite calluses and determination if the association is maintained in whole plants. Up to now we have been unable to accomplish plant regeneration from these calluses.

The use of genetic markers and nutritional requirements to establish complementary associations between different cell types should be a versatile procedure applicable to diverse experimental situations. The cellular association reported in this work might serve as a first step in the development of new or more general symbiotic relationships.

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Chromatin attachment to nuclear membrane of wheat pollen mother cells

THE cellular mechanisms through which homologues recognise each other before meiosis are unresolved. The problem is compounded in a polyploid species like *Triticum aestivum* which contains three diploid sets of genetically similar chromosomes¹. At meiosis, pairing normally takes place only between the fully homologous partners of a set, so that in spite of their close genetic similarity, homœologues do not synapse. The absence of homœologous pairing depends principally upon the presence of chromosome 5B (ref. 2) and probably derives from the operation of the *Ph* locus which is distally located on the long arm of that chromosome^{3,4}.

Recently there has been increased interest in premeiotic development in wheat⁵ and it has become clear that activities occurring during premeiotic interphase are critical to chromosome alignment^{6,7}. As such alignment might be effected by a structural mechanism we undertook a preliminary investigation of the ultrastructure of the wheat pollen mother cells (PMCs) at premeiotic interphase. Here we report our observation of fibrous material which seems to link chromatin to the nuclear membrane during premeiotic interphase, and discuss the possible significance of this material.

Plants of *T. aestivum* (var. Chinese Spring) were transferred to a constant environment at 20°C with continuous light, about 2 weeks before meiosis in leading tillers. When tillers reached the required developmental stage they were excised and transferred to the laboratory. The three anthers were quickly excised from each of several florets. One anther from each floret was fixed in 1:3 acetic acid and a Feulgen stained squash preparation prepared. Appropriate florets were identified from cytological examination of these squashes using the criteria previously described⁸. The remaining two anthers from such florets were fixed in 5% glutaraldehyde and prepared for ultrastructural studies. As the three anthers within a floret are approximately synchronous in development⁸, those used for ultrastructural studies should contain PMCs at the same stage as the Feulgen-stained samples. Nevertheless, to rule out possible mistakes due to variation between anthers, the stage of development was rechecked using thick and thin sections stained with methylene blue and osmium, respectively. The checks in squashed and sectioned preparations were based on the same criteria. A description of the sequence and appearance of the stages examined has been given previously⁵.

A distinct and novel feature of the ultrastructure of premeiotic cells was the presence of bundles of fibrillar material in the PMC nuclei. Individual fibres had a diameter of about 200 Å. Such fibrillar material was observed adjacent to the nuclear membrane apparently forming attachments at intervals between the membrane and chromatin masses (Fig. 1*a* and *b*). Bundles of fibrillar material were also found well within the nucleus. Figure 1*d* shows one such bundle about 1.2 µm long

and 0.2 µm wide apparently attached to chromatin at one end. Similar fibrillar material was not found in any other anther cells even though it was a general and constant characteristic of the PMCs. The exclusive presence of these fibres in the premeiotic but not in the adjacent somatic cells makes it likely that they are exercising some specific meiotic function.

The order of appearance of these fibrils relative to that of other meiotic nuclear structures is particularly relevant to speculation about their function. The relationship is illustrated diagrammatically in Fig. 2. The fibrils appear in the nuclei of premeiotic cells well before the appearance of any lateral elements and also before the investiture of the cells with callose, another distinctive characteristic of plant meiocytes. The presence of the fibrils overlaps the appearance of lateral elements and of the synaptonemal complex. The fibrils do not persist over the life time of the synaptonemal complexes. They were absent from sections of PMCs that had developed beyond 'stage 3'. At, or soon after 'stage 3', paracrystalline bodies were seen adjacent to the nuclear membrane (Fig. 1*e*). These may represent a step in the reorganisation or in the degradation of fibrillar material.

Some details of the changes occurring within the neighbourhood of the nuclear membrane are of interest in connection with the relationship of the nuclear membrane to chromosome synapsis, a relationship for which circumstantial evidence is available in a variety of studies^{8–10}. Before and during 'stage 1' a large proportion of the nuclear membrane was in contact with

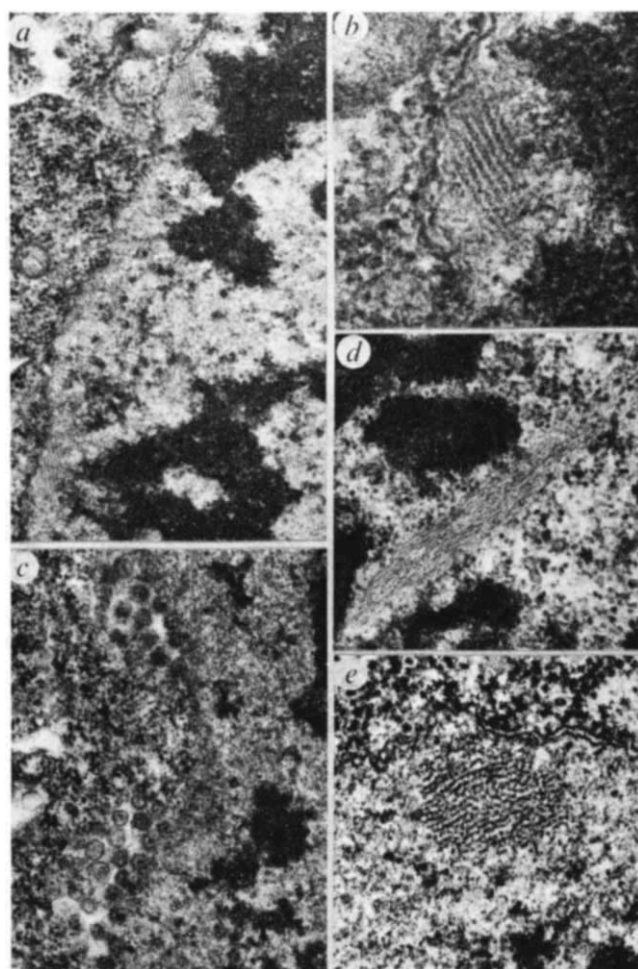


Fig. 1 Electron micrographs of PMCs from Chinese Spring wheat showing: *a*, fibrillar material located between the nuclear membrane and chromatin masses ($\times 28,700$) at 'stage 2'; *b*, a higher magnification ($\times 71,820$) of fibrillar material from *a*; *c*, pores in the nuclear membrane at 'stage 2' ($\times 28,700$); *d*, a large mass of fibrillar material located within the nucleus but away from the nuclear membrane ($\times 33,600$) and, *e*, a paracrystalline body adjacent to the nuclear membrane at 'stage 3' ($\times 57,400$).

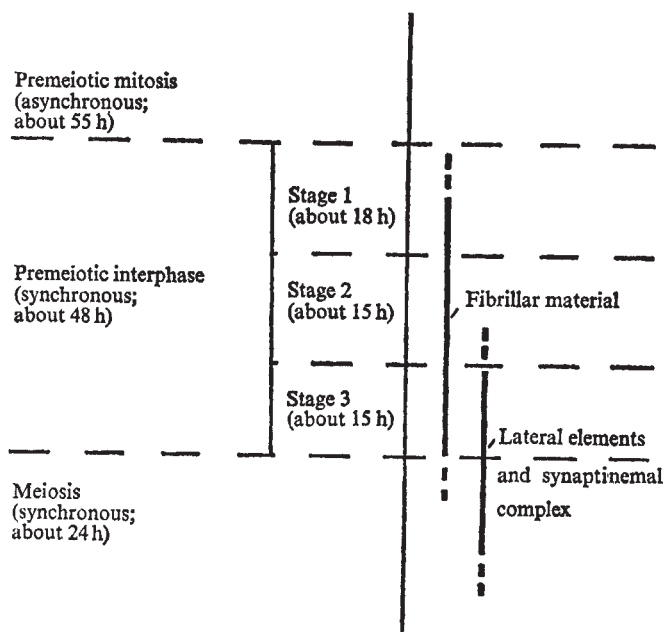


Fig. 2 The time and sequence of appearance of fibrillar material, lateral elements, and SC during premeiotic interphase and meiosis in PMCs of Chinese Spring wheat grown at 20°C.

chromatin either directly or through the fibrous material. Throughout this period the nuclear membrane had very few obvious pores. Early in 'stage 2' it became punctured by numerous pores (Fig. 1c) without, however, removing the fibrous material. Also, during 'stage 2' a zone about 2,000 Å wide containing little or no chromatin appeared next to the nuclear membrane (Fig. 1a and c). This zone, which differed in appearance from the rest of the nucleoplasm, extended around the whole circumference of the nucleus and contained in addition to the fibrillar material numerous structures, less defined but nevertheless sufficiently similar to suggest their functioning as agents of interaction between the nuclear membrane and chromatin.

The discovery of synaptonemal complexes revealed the nature of the mechanism that apparently stabilises meiotic chromosome pairing. The means by which homologues are suitably juxtapositioned before synaptonemal complex formation remain unknown, however. Some organisational framework would seem to be necessary for facilitating the prealignment of chromosomes for synapsis. We have found bundles of fibrillar material apparently forming long range connections with chromatin (Fig. 1d) and also linking chromatin to the nuclear membrane (Fig. 1c). It is noteworthy that the appearance of fibrillar material coincides with the interval when chromosome synapsis in wheat is sensitive to the action of colchicine⁶ or to low temperature⁷. Equally noteworthy is the fact that this interval begins before the initiation of synapsis and of lateral element, or synaptonemal complex formation. Colchicine has been shown to interfere with the normal functioning of the nuclear membrane during meiotic prophase in *Lilium*⁸ and, therefore, it may be significant that an appreciable fraction of the fibrils in wheat has been found associated with the nuclear membrane. We suggest, therefore, that the fibrillar material found only in PMCs at stages 1 to 3 may function in establishing or maintaining the spatial coorientation of chromosomes which is a prerequisite for normal meiotic pairing in wheat, and also that it may contain colchicine sensitive protein.

The appearance of fibrillar material before the first synaptonemal complex formation, and its disappearance soon after, would fit this hypothesis. If fibrillar material is responsible for long range links it would presumably cease to be useful once stable links are established between homologues unless the fibrils themselves become incorporated in the synaptonemal complex.

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Does the rete ovarii act as a trigger for the onset of meiosis?

ALTHOUGH transformation of oogonia to oocytes in the mammalian ovary and the subsequent formation of follicles have been much studied the factors which initiate meiosis remain obscure and the control of the formation of the granulosa layer is poorly understood.

Experimental results presented here indicate that the rete tubuli is important for the onset of meiosis and that the early formation of the granulosa layer is dependent on the attachment of the rete system to the ovary. Moreover, normal growth or maintenance of the ovarian tissue itself seem to be dependent on the extraovarian rete tubules.

The morphological similarity between the rete ovarii and the rete testis was already recognised in the last century¹. Both consist of tubules lined with cuboidal or cylindrical epithelial cells, which arise from the mesonephros. During early follicle formation a continuity between the tubes from the rete ovarii

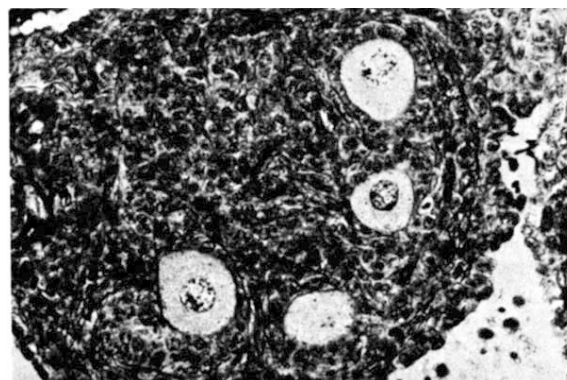


Fig. 1 Intact foetal mouse ovary (12 d post coitum) after inoculation in nude mouse for 14 d. Four apparently normal growing oocytes are seen ($\times 423$) (Stain: Azan).

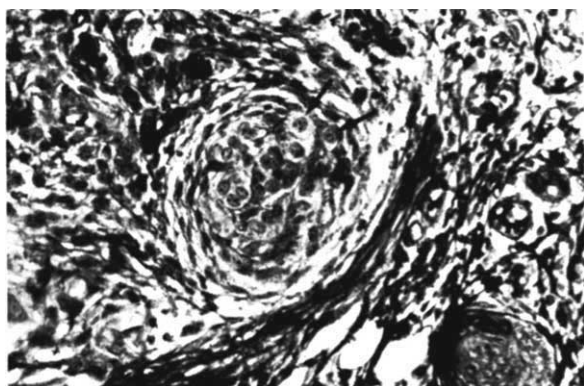


Fig. 2 Caudal part of a 12-d-old mouse ovary without the rete tubules after 14 d inoculation into nude mouse. The graft contains only oogonia (arrows) and no follicle cells. ($\times 423$) (Stain: Azan).

and the follicles has been seen²⁻⁶. Kölliker² and Byskov and Lintern-Moore⁶ proposed that cells of the rete ovarii contributed to follicle formation.

To test experimentally whether the onset of meiosis is dependent on the rete ovarii, we used ovaries from two groups of mouse embryos. As the transformation of oogonia to oocytes occurs in the mouse between the 13th and the 17th day of embryonic life^{7,8}, one group was aged 12 d *post coitum*, in which the rete tubuli had not yet invaded the gonads, but were lying entirely outside the ovary as extraovarian rete and the meiotic prophase had not started; in the second group, aged 15 d *post coitum*, the rete was present within as well as outside the ovaries and the major part of the germ cells had entered the meiotic prophase. Under a dissecting microscope three ovaries in each group of six were divided into a cranial part with the adherent extraovarian rete tubules and mesonephric remnants and a caudal part of 'pure' gonadal tissue, without extraovarian rete tubules. The three other ovaries in each group were left intact. In order to study the transformation of oogonia to oocytes in gonads with and without the presence of the extraovarian rete tubules the intact ovaries and the cranial and

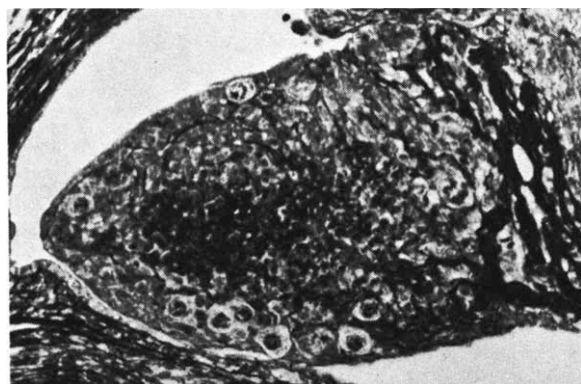


Fig. 3 Caudal part of a 15-d-old mouse ovary without the rete tubules after 11 d implantation in nude mouse. Some oocytes are seen in the periphery of the graft. No follicles have formed. Necrotic changes are seen in the centre of the graft. ($\times 423$) (Stain: Azan).

caudal parts of ovaries were inoculated subcutaneously into adult female mutant nude mice with aplasia of the thymus (strain BALB/c Nu/Nu). This method of grafting has already been described⁹.

The embryonic ovarian tissue was removed from the nude mice and prepared for histological study after 14 and 11 d respectively. At removal the grafted gonads corresponded to a 5-d-old mouse ovary.

Intact grafted ovaries and the cranial part of the ovary with

the rete tubules attached, both taken from 12-d-old fetuses, contained small and medium sized follicles at 'day 5' (Fig. 1). Their oocytes were indistinguishable from oocytes seen in a normal 5-d-old mouse ovary. The granulosa layer of the follicles was more irregular than normally seen at day 5 with many finger-like extensions to other follicles or to the rete system. Necrotic changes did not occur in these transplants.

The caudal part of the 12-d-old embryonic ovaries without the extraovarian rete tubules grafted for 14 d contained only oogonia (Fig. 2). Oocytes and follicles were never seen. The oogonia were surrounded by undifferentiated fibroblast-like cells. In two of the three grafts necrotic changes were observed. In contrast to this the cranial part of the ovary with the extraovarian rete tubules attached contained oocytes as well as follicles after inoculation for 14 d.

All the ovaries grafted from the 15-d-old embryos, both with and without the extraovarian rete tubuli attached, contained oocytes after implantation for 11 d. Intact ovaries as well as the cranial part of ovaries developed follicles, which corresponded in size to those found in a 5-d-old mouse. None of those grafts showed necrotic changes. The caudal part of the ovary without the rete tubules consisted only of a mass of undifferentiated cells with oocytes lying closely together in group at the periphery (Fig. 3). Also in these rete-depleted grafts necrotic changes were seen in two cases. No follicular formation had occurred.

Recent ultrastructural studies show that the extraovarian rete cells are very active holocrine secreters. Whether the effect of the rete system depends on direct cellular contact with the germ cells or on a trigger component secreted from the rete cells remains still uncertain.

Preliminary studies of implantation of ovaries from rabbits and hamsters support our results. The rete ovarii is essential for the start and maintenance of meiosis and for further ovarian development.

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How selective is high affinity uptake of GABA into inhibitory nerve terminals?

It is now well established that brain slices and homogenates possess distinct high affinity uptake systems for all the major transmitter candidates or their immediate precursor molecules^{1,2}. In the case of GABA and glycine the application of electron microscopic autoradiography to such *in vitro* preparations has revealed discrete populations of nerve terminals as the major site of amino acid uptake³. In brain slices or homogenates, however, nothing is known about the functional or

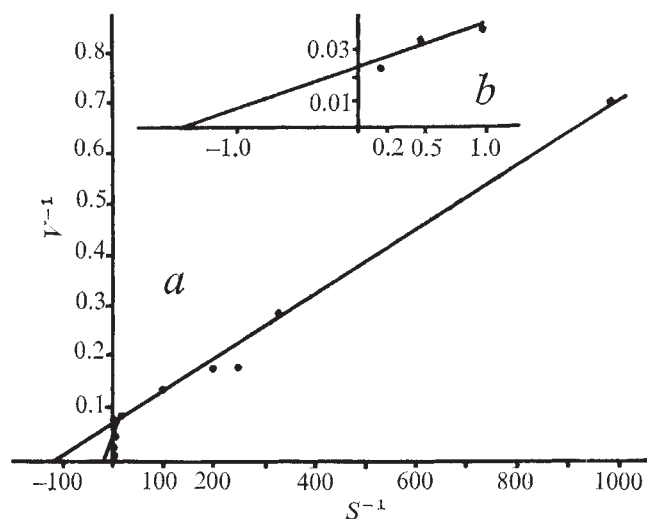


Fig. 1 Lineweaver Burk plots from a single experiment to show high and low affinity uptake of ^3H -GABA into cerebellar glomerulus particles. The kinetics of the uptake were characterised by two straight lines, the shallower of which described a high affinity system with an apparent K_m and V_{max} of 9.6 μM and 15.6 nmol per mg protein per 10 min respectively and the steeper (a, shown in more detail above) a low affinity system with a K_m and V_{max} of 780 μM and 46.2 nmol per mg protein per 10 min (S = GABA concentration in nM and V = velocity in nmol per mg protein per 10 min). The glomerulus particles (320 μg protein) were incubated at 37°C for 10 min in 1 ml of an isotonic salt medium, buffered with Tris-HCl, pH 7.4 and containing 32 mM of glucose and 10 μM amino-oxyacetic acid. The size of the inulin space and ^3H -GABA concentration of the particles was estimated by the method of Levi and Raiteri¹³. The experimental points are the means of duplicate estimates and the lines were plotted by computer analysis.

anatomical identity either of the labelled or the unlabelled populations of terminals. In other words, it has not hitherto proved possible to confirm that each high affinity uptake process for any particular transmitter substance is present exclusively in those nerve terminals in the brain known from independent neurophysiological studies⁴ to release that substance.

Fortunately, the newly perfected technique for isolating large pieces of the cerebellar glomeruli with well preserved ultrastructure (glomerulus particles)⁵⁻⁷, now offers a preparation suitable for testing the two fundamental propositions of this hypothesis (at least for the putative transmitter GABA) namely that the high affinity uptake system is exclusively present in recognisable inhibitory endings which are known to utilise GABA as their transmitter substance and that the uptake is completely absent from other categories of nerve terminals. The inhibitory nerve terminals of the cerebellar glomeruli arise from Golgi cell axons⁸, and form one of the two inputs on to the granule cell dendrites. These inhibitory terminals are adjacent to the excitatory endings of the mossy fibres, which can easily be distinguished from the Golgi cell axon terminals by their large size and other ultrastructural features^{9,10} (Fig. 2).

The presence of a high affinity uptake process for ^3H -GABA in the glomerulus particles was demonstrated in three separate experiments in which glomeruli were incubated in the presence of a wide range of GABA concentrations (1–5,000 μM) (see Fig. 1). The K_m values obtained (between 7 and 12 μM) were almost identical to those reported in slices of cerebral cortex⁹. In addition, ^3H -GABA was also taken up by a second low affinity uptake process which was characterised by a K_m of 780 μM . The tissue components responsible for the high affinity uptake process were identified by electron microscopic autoradiography carried out on glomerulus particles incubated in the presence of a low concentration of ^3H -GABA (2.5 μM), thus avoiding any appreciable uptake by the low affinity process (Fig. 2). In spite of incubation in media containing

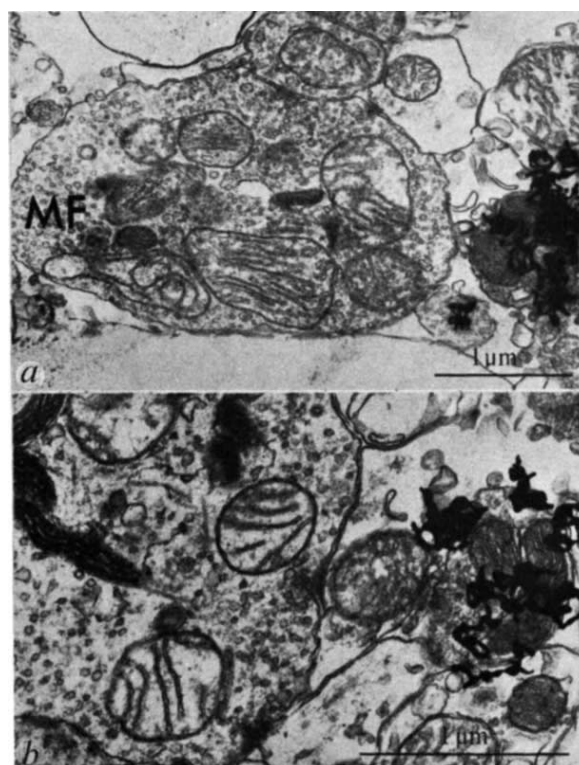


Fig. 2 a, Electron microscopic autoradiograph of a long-term particle showing the marked contrast between the mossy fibre terminal (MF) which is completely devoid of silver grains and the small Golgi axon on the right of the picture which is overlaid by an intense cluster of silver grains. b, A small portion of another glomerulus particle in which the unlabelled mossy fibre terminal and granule cell dendrite is shown to be in close morphological proximity to the intensely labelled Golgi axon terminal. The glomerulus particles (about 1–2 mg protein derived from six rat cerebella) were incubated in the presence of 2.5 μM ^3H -GABA (specific activity 10 Ci mmol⁻¹) at 37°C for 6 min in 1 ml of buffered saline. After incubation, the glomeruli were centrifuged, washed, fixed in 5% glutaraldehyde and processed for autoradiography³.

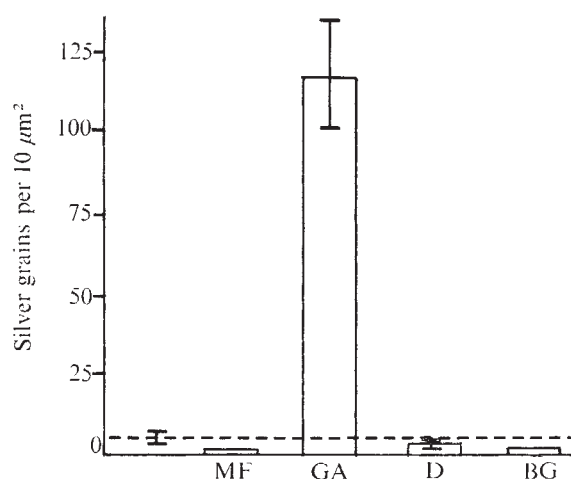


Fig. 3 Distribution of silver grains over electron microscopic autoradiographs of isolated cerebellar glomeruli labelled with ^3H -GABA. The histogram (mean $\pm$ s.e.) from an analysis of 15 different electron micrographs, each containing one complete glomerulus particle, to show the silver grain density as a function of area for the various identifiable components, the mossy fibre terminals (MF), the Golgi axons (GA), the granule cell dendrites (D) and the background (BG). The dotted line shows the average density of silver grains per micrograph, each of which was 8×10 inches and represented an area of 60 sq μm .

saline, the fine structure of the glomerulus particles was well preserved.

Intense clusters of silver grains resulting from the accumulation of ^3H -GABA were found almost exclusively over small nerve terminals which closely resemble the Golgi axon terminals characterised by Palay and Chan-Palay¹⁰ in sections from *in situ* fixed adult rat cerebellum. Such grain clusters were never seen over the adjacent mossy fibre terminals. To emphasise the selectivity of the uptake into inhibitory, as opposed to excitatory terminals, a statistical analysis of the silver grain distribution was carried out on 15 electron micrographs, each of which contained a complete glomerulus particle (Fig. 3). The silver grain density over the Golgi axons was more than 20 times higher than the average density per micrograph and more than 100 times greater than the grain density over the mossy fibre terminals. The postsynaptic granule cell dendrites, like the excitatory terminals, were virtually devoid of silver grains.

As well as confirming the hypothesis set out in the introduction these results taken in conjunction with the previously reported concentration of glutamic acid decarboxylase in the glomerulus particles^{6,7} and its presence in Golgi axon terminals *in situ*¹¹ suggest that these inhibitory terminals retained their *in vivo* properties. This view is strongly supported by a parallel study¹² in which a microinjection of the GABA analogue ^3H -2, 4-diaminobutyric acid was shown to cause an identical distribution of silver grains to occur *in vivo*.

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Glutamate receptors in the rat central nervous system

ALTHOUGH it is widely accepted that glutamate may be a significant central transmitter (for reviews, see refs 1 and 2), definitive neuropharmacological evidence at the synaptic level has not been forthcoming, and studies with proposed glutamate antagonists³⁻⁶ have yielded anomalous results. On the basis of biochemical evidence, however⁷, glutamate fulfils many of the criteria expected of a neurotransmitter; in particular, nerve

endings⁸ and glia^{9,10} possess high-affinity transport systems for glutamate, which it is thought, in common with other amino acid neurotransmitters, are responsible for terminating its synaptic actions. For these high-affinity systems to function, there is a stringent requirement for sodium¹¹, which may be involved in the initial binding phase at the reuptake site, prior to transport¹²⁻¹⁴. Until recently, very little attention has been paid to the possibility of investigating directly the biochemical properties of postsynaptic amino acid receptors. Young and

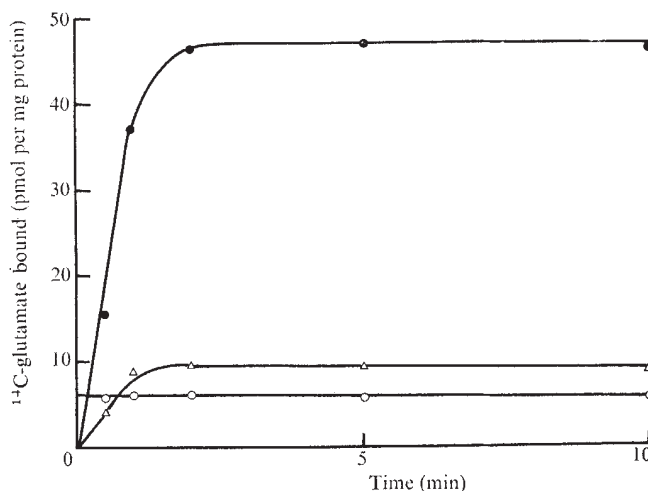


Fig. 1 Time course of binding of ^{14}C -glutamate to synaptic membranes. Male Sprague-Dawley rats were decapitated and cerebral cortices homogenised in 20 vols ice-cold 0.32 M sucrose (Teflon homogeniser, 0.25 mm clearance, 800 r.p.m.) and centrifuged at 1,000g for 10 min. The supernatant was then centrifuged for 20 min at 17,000g to give a crude mitochondrial pellet (containing mainly myelinated axons, mitochondria and synaptosomes). The pellet (P_2) was resuspended in 1.2 ml 0.32 M sucrose, which was layered on to a discontinuous gradient consisting of: 4 ml 0.8 M sucrose, 4 ml 1.2 M sucrose and 2 ml 1.5 M sucrose. Gradients were centrifuged in 6×15 ml swing-out heads for 60 min at 112,000g. The synaptosomes were collected at the 0.8–1.2 M sucrose interface, lysed with 20 vols ice-cold distilled water in a glass homogeniser and the homogenate centrifuged for 20 min at 9,000g. The supernatant and the soft pellet were removed and recentrifuged at 50,000g for 60 min, the resulting pellet resuspended in Tris-Krebs buffer, pH 7.4 (containing 50 mM Tris substituted for bicarbonate, and either 115 mM sodium chloride or 230 mM sucrose) and aliquots taken for protein estimations¹⁷. Membrane suspensions (6.0 – 6.5 mg protein ml^{-1}) were stored at -30°C , no significant loss of binding activity was detected after 2 months.

Synaptic membranes (0.31 – 0.35 mg protein ml^{-1}) were incubated in 0.5 ml Tris-Krebs medium (with or without sodium⁺) at 25°C and containing 1.75×10^{-6} M L- ^{14}C -glutamate (Amersham) for periods of time from 30 s to 10 min. Membranes were recovered by rapid (approx 5 s) vacuum filtration through Whatman GF-C glass fibre filters followed by rinsing with 5 ml Tris-Krebs. Filters were transferred to vials and the tissues digested with 0.4 ml Soluene-100 (Packard) prior to addition of fluor for liquid scintillation counting. To correct for binding of ^{14}C -glutamate at sites other than for uptake and the postsynaptic receptors (that is, nonspecific binding), the binding of ^{14}C -glutamate was studied in the presence of a large molar excess of unlabelled L-glutamate. This nonspecific binding was not saturable and increased proportionately with increasing ^{14}C -glutamate concentration. In all experiments to determine specific glutamate binding, this nonspecific component was subtracted. The effect of synaptic membrane protein concentration on specific ^{14}C -glutamate binding was also investigated and was found to be linear over the range studied (0.2 – 0.5 mg protein ml^{-1}). ●, Specific ^{14}C -glutamate binding in the presence of sodium; △, specific ^{14}C -glutamate binding in sodium-free medium; ○, nonspecific ^{14}C -glutamate binding (blank values, ^{14}C -glutamate bound to filters in absence of tissue subtracted from each value) determined in the presence of unlabelled glutamate (5×10^{-3} M). Results are means of quadruplicate determinations; s.e.m. all $\leq 10\%$.

Snyder¹⁵ have demonstrated that strychnine, a potent and selective antagonist of glycine-induced hyperpolarisations of spinal neurones, binds specifically to a component of synaptic membranes; this is probably the physiological glycine receptor since strychnine has negligible affinity for the glycine high-affinity uptake system. The specific γ -aminobutyric acid (GABA) antagonist, bicuculline, has also been found to competitively inhibit GABA binding to synaptosomes, in which the uptake site had been inactivated by chlorpromazine¹⁶. In both these cases, binding to the postsynaptic receptor was not affected by the absence of sodium.

I have therefore examined the possibility that in the absence of sodium, the characteristics of the still hypothetical, specific glutamate receptor on synaptic membranes might be investigated.

Figure 1 shows the time course of specific ^{14}C -glutamate binding at 25°C and, in common with the binding of strychnine to glycine receptors, nonspecific binding was not time dependent (essentially instantaneous), whilst the specific binding was rapid, with a plateau after 2 min. Half maximal binding occurred within 30 s, both in the presence and absence of sodium. When binding was studied at 0°C, maximal binding was not apparent for 8 min. All further binding studies were carried out with a 5 min incubation period.

Kinetic characteristics of specific glutamate binding were then examined under two experimental conditions: (1) in normal Tris-Krebs medium containing N-methyl-DL-aspartate (10^{-3} M), a highly potent glutamate agonist¹⁸ with no affinity for the glutamate uptake system¹⁹⁻²¹; and (2) in sodium-free Tris-Krebs medium. Such a scheme should theoretically permit assessment of binding to the uptake and receptor sites individually. Binding was measured over a restricted range of ^{14}C -glutamate concentrations (4×10^{-7} – $8.7 \times 10^{-6}\text{ M}$) and

Table 1 Pharmacological antagonism of specific ^{14}C -glutamate binding

Treatment	+ Na ⁺		Tris-Krebs medium – Na ⁺	
	Specific binding*	Inhibition† %	Specific binding	Inhibition %
Control	44.00 ± 0.41	—	8.83 ± 0.41	—
D-glutamate	40.86 ± 0.20	5	7.83 ± 0.13	11‡
L-aspartate	3.65 ± 0.16	79	3.25 ± 0.20	63§
DL-homocysteate	19.99 ± 1.43	42§	3.30 ± 0.99	63§
Glutamic acid diethyl ester	38.17 ± 0.50	0	3.52 ± 0.27	60§
p-Chloromercuriphenylsulphonate	34.03 ± 0.44	18‡	6.46 ± 0.33	27‡

Synaptic membranes were incubated in Tris-Krebs medium for 5 min with L-U- ^{14}C -glutamate and one of the above substances at a concentration of 10^{-3} M .

*Specific binding in pmol per mg protein.

†Percentage inhibition in the presence of Na⁺ corrected for binding component to receptors (–Na⁺ alone).

Means ± s.e.m. of quadruplicate determinations; significance of difference from control in each group (that is, +Na⁺ & –Na⁺) by student's *t* test.

‡*P* < 0.05.

§*P* < 0.005.

||*P* < 0.001.

the double reciprocal plots (Fig. 2) reveal apparent dissociation constants (*pK*s) of $4.0 \times 10^{-6}\text{ M}$ and $8.3 \times 10^{-6}\text{ M}$ for the uptake and receptor sites respectively.

If there are subtle configurational differences^{5,19} between the postsynaptic glutamate receptor and the presynaptic high-affinity uptake site, an investigation of specific ^{14}C -glutamate binding to synaptic membranes in the presence (binding to both sites) and absence (binding to receptors only) of Na⁺ should readily provide the means of distinguishing between glutamate agonists and the highly elusive glutamate antagonists. Acidic amino acids such as N-methyl-DL-aspartate and D-homocysteate, with strong excitant actions on central neurones, are not substrates for the high-affinity uptake system¹⁹⁻²¹—a property shared only with the five compounds which have been suggested as effective antagonists of glutamate-induced and some synaptic excitations in the CNS, that is, glutamic acid diethylester^{4,5}, α -methyl-DL-glutamic acid³, 1-hydroxy-3-aminopyrrolid-2-one (HA-966)⁶, 2-methoxyaporphine⁵ and L-methionine-DL-sulphoximine⁵. In contrast, medium excitants, such as L-glutamate, L-aspartate, L-cysteate and L-homocysteate, are all potent blockers of high-affinity glutamate uptake. Table 1 shows the effects of Na⁺ on specific L-glutamate binding and how this can be modified by the presence of some glutamate congeners. Binding is stereospecific as evidenced by the lack of competition by D-glutamate, which is a weak excitant with minimal effects on uptake. L-aspartate produced a marked inhibition of binding at both sites, while DL-homocysteic acid (DLH) showed a greater effect at the glutamate receptor than at the reuptake site. DLH is considerably more potent as a neuronal excitant than is either L-glutamate or L-aspartate¹⁸, and my results suggest that this may be attributable to the lesser ability of DLH to bind to the uptake site, than either glutamate or aspartate, since its affinity for the glutamate receptor appeared to be equivalent to that of L-aspartate. Glutamic acid diethylester, one of the more effective glutamate antagonists, had no effect on the specific binding of ^{14}C -glutamate at the uptake site, but produced a highly significant inhibition of binding at the receptor site. Finally p-chloromercuriphenylsulphonate, a substance which at low concentrations potentiates the synaptic actions of both excitant and inhibitory amino acids, possibly by inhibiting their reuptake²², produced some reduction of specific glutamate binding at both sites. These effects were probably nonspecific, since mercurials combine extensively with the sulphhydryl groups of membrane components^{23,24}.

These results support the idea that specific glutamate binding to its postsynaptic receptor may be distinguished from binding

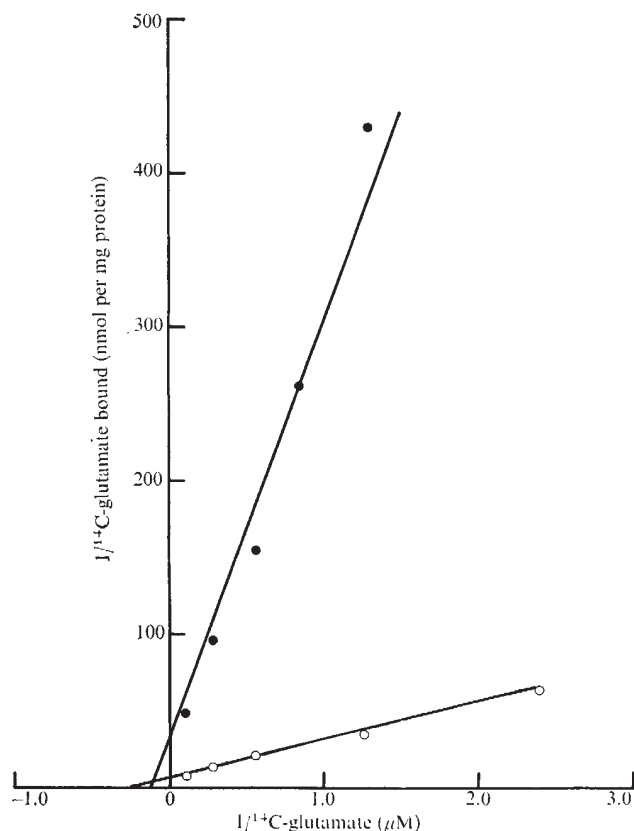


Fig. 2 Lineweaver-Burk plots of ^{14}C -glutamate binding to cortical synaptic membranes in Tris-Krebs medium containing N-methyl-DL-aspartate (10^{-3} M) (○) and in sodium-free medium (●). Lines of best fit by regression analysis. Each point is the mean of quadruplicate determinations; s.e.m. all < 10%.

to its uptake site by its independence of sodium. I am now investigating the effects of a wide range of potential glutamate antagonists on the specific binding of glutamate, and attempts will be made to isolate and characterise these binding components, as has been done for the insect glutamate receptor²⁵.

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Table 1 Chromosome composition of VA2 x mouse nervous system hybrid cell lines

Cell line	Telocentric	Mean chromosome no. (Range)	
		Biarmed	Total
Parent			
VA2	6 (1-11)	68 (52-79)	74 (66-83)
Normal mouse	40	0	40
Expected	46	68	114
Hybrid			
VMPH12	36 (32-38)	87 (78-93)	123 (115-131)
VMPOC18E	31 (23-36)	50 (44-56)	81 (72-89)
VMPE18F	27 (18-33)	58 (49-67)	85 (67-98)
VMPOC15M	26 (19-34)	51 (45-63)	77 (67-89)
VMPH14D	22 (18-27)	89 (80-101)	110 (98-124)
VMPH18	18 (10-27)	84 (74-94)	102 (96-117)
VME15	13 (6-18)	96 (77-106)	109 (93-121)
VME10	11 (5-16)	63 (56-76)	74 (65-84)
VMPH28G	9 (5-17)	64 (81-49)	73 (56-93)
VMSC10	8 (5-11)	82 (66-92)	90 (74-107)
VMSG5	5 (4-9)	84 (70-99)	89 (81-103)

Cells were grown in modified F12⁵ medium (F) or Dulbecco's modified Eagle's medium (D), supplemented with 5% foetal calf serum and, as indicated, HAT (FHAT, DHAT), in an atmosphere of 5-10% CO₂, 90-95% air with 95% relative humidity, at 37°C in Falcon plastic ware. Parental VA2 cells after growth in 100 µM 6-thioguanine-supplemented medium formed < 1 × 10⁻⁷ colonies per inoculated cell in FHAT, while normal mouse nervous system cells formed 1-5 × 10⁻⁷ colonies per inoculated cell in FHAT. Trypsinised, washed, VA2 cells (3 × 10⁶) were mixed with normal mouse cells (10⁴-5 × 10⁶) in serum-free medium (1 ml) and fused with 200-500 haemagglutinating units (0.03% chick red blood cells) of β-propiolactone-inactivated Sendai virus, 10 µg DNase I, as before⁴. Washed, fused cells were either diluted immediately in medium into many 100 mm dishes or seeded into one dish and then trypsinised and distributed 24 h later. All cells were transferred to FHAT medium 24 h after fusion, fed every 4 d with FHAT, and hybrid colonies were isolated 18-24 d later as independent mating events by trypsinisation in penicillins (Fischer Scientific Co.). Many lines were recloned before testing (indicated by letter suffixes of clone name). Cells were subcultured by trypsinisation approximately once a week and intermittently stored in the vapour phase of a N₂ freezer with 7.5% dimethylsulphoxide (DMSO) or 5% DMSO and 5% glycerol-supplemented medium. Chromosome preparations were prepared by arresting logarithmic phase cells (50-100 doublings after fusion) in 0.02 µg ml⁻¹ colcemid for 4 h, swelling in 75 mM KCl, air drying and then scoring 20-40 metaphases per cell line using a camera lucida attachment on a Wild microscope. Embryonic mouse brain (18 d *in utero*) was dissociated, cultured in DHAT for 2 weeks, and then scored. Eighty-six per cent of the cells had 40 chromosomes, 2% had 37, 6% had 50, and 6% had 80 chromosomes.

hypoxanthine phosphoribosyltransferase (HPRT) and thus unable to grow in HAT-supplemented medium³. A suspension of parental mouse cells was obtained by dissecting specific anatomical regions of C57BL/6 or NIH/Swiss-GP 15-18 d *in utero* embryonic mouse nervous systems, pooling the same regions from one litter, and then dissociating cells in trypsin, collagenase and DNase⁴. Cells were used for fusion directly (VM series) or incubated overnight in complete medium and the non-attached cells collected (VMP series).

Using Sendai virus (legend of Table 1) hybrid cell lines were generated repeatedly in fusions between human VA2 cells and cells freshly derived from the embryonic mouse, rat or Chinese hamster nervous system resulting in the same pattern of reverse chromosome segregation; however, only data on human-mouse hybrids are presented here. Approximate yields were 3 × 10⁻⁵ hybrid colonies per VA2 parent and 2 × 10⁻⁵ per normal rodent nervous system cell put into the fusion. The cells appeared fibroblastic, not similar in morphology to mouse neuroblastoma cells⁶, and all were negative when stained for acetylcholinesterase⁶ (1,850 colonies scored). The population doubling times (and plating efficiency) of hybrid cell lines varied widely and representative samples were studied.

In the VA2 × mouse nervous system cell lines the number of biarmed and presumably human chromosomes was greater than the human diploid value (2n = 46) and similar to that of VA2 (Table 1). In contrast, the number of telocentric (mouse)

Human x mouse hybrid cells segregating mouse chromosomes and isozymes

THE usual pattern of chromosome segregation in interspecific human × mouse somatic cell hybrids is retention of mouse and loss of human chromosomes¹. In attempts to generate hybrid strains expressing differentiated functions, we have fused an actively dividing human cell line with freshly dissociated, highly differentiated cells from the mouse embryonic nervous system. The majority of the resulting hybrid strains retain human while losing mouse chromosomes and isozymes and thus provide a new class of hybrid cell for analysis of gene linkage and regulation.

The human fibroblast parent used was WI-18-VA2 (VA2)², a SV40-transformed clonal derivative of WI-18, deficient in

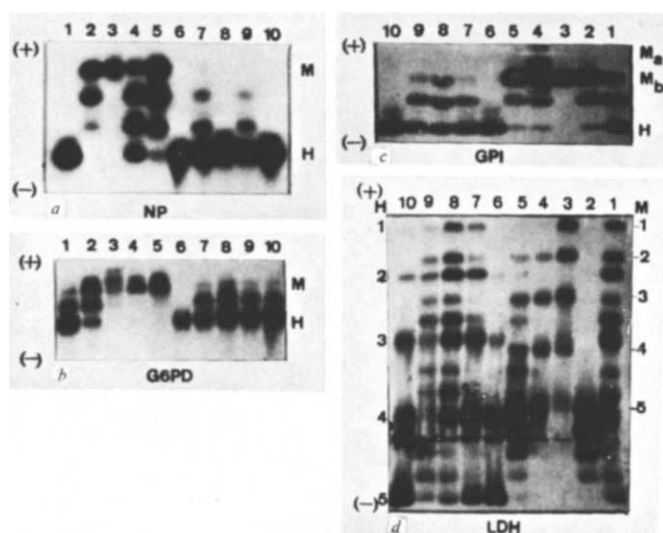


Fig. 1 Zymograms for: (a) nucleoside phosphorylase; (b) glucose 6-phosphate dehydrogenase; (c) glucose phosphate isomerase; (d) lactic acid dehydrogenase. M, mouse; H, human pattern. In (c), M_a and M_b indicate migration of mouse GPI-1A, and GPI-1B isozymes and the hybrid cell in slot 4 was derived from fusion of a VA2 cell to a mouse cell (NIH/Swiss GP) heterozygous (GPI-1AB) at this locus. In (d), 1, 2, 3, 4, 5 indicate mobility of human or mouse LDH isozyme bands alone. Cell lines were: 1, VMPTH12H; 2, VB3A; 3, C57BL/6 mouse embryo brain; 4, VMSG11; 5, VMSC24, 6, VA2; 7, VMPTH14D; 8, VMPTH12; 9, VMPOC15M; 10, VMPTH28G. Note that hybrid lines in slots 4 and 5 were preselected for retention of telocentric and loss of biarmed chromosomes, and thus represent an uncommon class while slot 2 is a VA2 × L-cell hybrid.

chromosomes was less than the mouse diploid value ($2n = 40$). Because some human D and G group chromosomes in VA2 may be confused with mouse telocentric chromosomes, the mouse chromosomes in these hybrid cells may have been overestimated. Karyological analysis by quinacrine-mustard chromosome banding shows that translocation of mouse chromosomes does not occur frequently enough to interfere with these estimates (our work in preparation).

Sixty to one-hundred population doublings after fusion, the hybrid strain phenotypes for 16 isozymes were determined (Table 2, Fig. 1). The genes for the human isozymes studied have been assigned to specific human chromosomes including: 1, 2, 6, 7, 11, 12, 14, 18, 19, 20, 21 and X¹³. Most of the hybrid lines continued to express all the human isozymes but failed to express certain of the mouse isozymes. A few remained balanced, expressing all the human and mouse isozymes tested. These balanced hybrid lines had fewer than 40 telocentric chromosomes. In hybrid cell lines with reduced numbers of telocentric chromosomes, the human isozymes exhibited equal or greater staining activity than the homologous mouse enzymes which contrasts with previous work with human × mouse hybrids (slot 9, Fig. 1). Heteropolymeric isozyme bands of intermediate mobility were noted for PEP A (DIP-2), cytoplasmic GOT, LDH A, MDH 1, GPI, IPO-dimeric, NP, IDH1, and G6PD in these hybrids as reported for other human × mouse hybrids¹³⁻¹⁵, which supports the homology of the markers for scoring purposes. A few hybrid strains retained telocentric and lost biarmed chromosomes (less than 5% of the total lines tested) and these segregated human isozymes (Table 3). In contrast, more than 50 VA2 × mouse L-cell hybrids made by us have segregated human while retaining mouse markers, suggesting that the SV40 genome in VA2 is not the main determining factor in loss of mouse chromosomes.

Table 2 Expression of mouse isozymes in VA2 × mouse nervous system hybrid cells (human isozymes always expressed)

Cell line	ADA	GPI	LDHA	TRIP-1	MDH1	IPO-A	PGM2	DIP-2	GOT	HK	NP	DIP-1	MPI	ID-1	LDHB	G6PD	HAT
C57BL/6 brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VA2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VMPOC15M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VMPOC18R	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VMPOC10D	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VMPOC15F	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VMPE18F	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VMPOC18E	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VMPTH14C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VMPTH14D	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VMPTH12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VMPTH18	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VMPTH28B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VMPTH28C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VMPTH12H	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VMPTH28G	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VME10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VME15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VMSC10	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VMSG4	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VMSG6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VME6	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VMSC13	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VMSG5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Cells were shifted from F to D medium with or without HAT supplementation depending on their ability to grow in HAT. For unexplained reasons, not all hybrid cell lines were capable of growth in DHAT after growth in FHAT (plating efficiency $< 10^{-5}$) in spite of the ability of VA2 to grow well in either F or D medium. The cell lines were free of mycoplasma contamination by bacteriological analysis (Microbiological Associates). Cells at confluence were washed three times with saline D₁ supplemented with 5.6 mM glucose, and 59 mM sucrose⁶ sonicated at 4°C in 50 mM KPO₄, pH 6.8 (total homogenate protein concentrations⁷, 10–20 mg ml⁻¹) frozen on dry ice, stored under the vapour phase of a N₂ freezer. Brains from C57BL/6 6-week-old males, or 16–18-d *in utero* embryos were processed similarly. Thawed homogenates were centrifuged (30,000g, 30 min, 4°C) and 250–500 µg of supernatant fluid protein was used per slot in a Buchler vertical starch gel electrophoresis using Electrostar (Otto Hiller) and isozymes were scored by standard procedures⁸⁻¹²: lactic dehydrogenase A and B (LDH A, LDH B); peptidase B (PEP B, mouse TRIP-1); peptidase C (PEP C, mouse DIP-1); peptidase A (PEP A, mouse DIP-2); glucose 6 phosphate dehydrogenase (G6PD); indophenol oxidase dimeric (IPO-A); mannose phosphate isomerase (MPI, mouse MPI1); nucleoside phosphorylase (NP); hexokinase (HK); adenosine deaminase (ADA); cytoplasmic glutamate-oxaloacetate transaminase (GOT1, mouse GOT); glucose phosphate isomerase (GPI); isocitrate dehydrogenase (IDH1, mouse ID-1); cytoplasmic NAD dependent malate dehydrogenase (MDH1); phosphoglucomutase 1 (PGM₁, mouse PGM₂). LDH B is activity of band 1 (see Fig. 1d) relative to VA2. HAT column in the tables indicates the ability of cell line to grow in HAT medium. Mouse isozyme nomenclature, when available, is used in this table. N = No activity; dash = uncertain.

Table 3 Expression of human isozymes in VA2 × mouse nervous system hybrid cells (human isozymes not always expressed)

Cell line	ADA	GPI	LDHA	PEPB	MDH1	IPO-A	PGM1	PEPA	GOT1	HK	NP	PEPC	MPI	IDH1	LDHB	G6PD	HAT
VMSC24	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VMSG11	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VB3A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VMSC14	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+
VMSC16	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+
VMSG2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

These hybrid lines were preselected from over 100 VA2 × mouse nervous system hybrids for retention of telocentric and loss of biarmed chromosomes. In these few lines, the mouse isozymes were always expressed and the (+) and (−) symbols refer to the expression of human isozymes. VB3A = VA2 × B82 (mouse L cell) hybrid line is presented for comparison as an example of a VA2 × mouse tissue culture line. Human isozyme nomenclature is used in this table.

Hybrid cells retaining human and losing mouse chromosomes provide information for mouse linkage studies. Our data from VA2 × mouse nervous system hybrids suggested associations of mouse isozymes (Tables 2 and 4). Mouse GPI, associated with mouse linkage group (LG) I¹¹, exhibited an apparent synteny with LDH A and TRIP-1 (human nomenclature, PEP B). In other experiments, Chinese hamster fibroblast × normal mouse spleen hybrids segregating mouse chromosomes and isozymes have yielded a few examples of asyteny of expression of mouse GPI, TRIP-1, and LDH A (in preparation). Pitfalls exist in somatic cell linkage analysis including non-random segregation of chromosomes¹⁶. In fact, since some isozymes were expressed more frequently than others, our data suggest non-random segregation of mouse markers in this system. Synteny data should be considered tentative and the presence or absence of specific mouse chromosomes must be demonstrated to be correlated with specific mouse isozymes.

Eight hybrids between VA2 (HPRT−) and mouse nerve tissue (HPRT+) that had lost telocentric chromosomes were removed from HAT medium about 40 doublings after fusion,

grown in medium F for forty doublings, and then found to be unable to grow in HAT medium. When tested for G6PD, apparent synteny was noted between the ability to grow in HAT and expression of mouse G6PD. It will be interesting to determine if mouse HPRT and G6PD are correlated with the presence of the mouse X chromosome as they are with the human X chromosome^{13,17}.

Several examples of asyteny of mouse isozyme expression, generated from the data in Table 2, are summarised in Table 4. With the exceptions of GPI, LDH A and TRIP-1, the other mouse isozymes appeared to be asytenic with each other. Thus, these isozymes probably mark 12 of 19 mouse autosomes. One case is perplexing and emphasises the need for caution in interpreting the results. DIP-1 and IDH-1 have both been assigned to LG XIII in the mouse¹¹. Yet, three instances of asyteny were detected in hybrid clones (Table 4). In all cases, DIP-1 was expressed while IDH-1 was not, possibly due to weak staining of mouse IDH-1.

Synteny of human PGM1 and PEP C and their correlation with the presence of chromosome 1, IDH and MDH1 with

Table 4 No. of VA2 × mouse nervous system hybrid strains showing asyteny for mouse isozymes (N = 22)

	HAT	G6PD	GPI	LDHA	TRIP-1	ADA	PGM2	MDH1	IPO-A	DIP-2	GOT	HK	NP	DIP-1	MPI	ID-1
G6PD	0															
GPI	6	6														
LDHA	6	6	0													
TRIP-1	6	6	0	0												
ADA	6	6	3	3	3											
PGM2	1	1	3	3	3	1										
MDH1	4	4	2	2	2	4	3									
IPO-A	2	2	2	2	2	4	3	2								
DIP-2	5	5	5	5	5	5	4	5	3							
GOT	9	9	7	7	7	9	8	7	9	8						
HK	6	6	6	6	6	8	7	4	6	7	3					
NP	9	9	7	7	7	9	8	5	7	7	4	1				
DIP-1	12	12	9	9	9	9	8	7	4	6	2	3	4			
MPI	10	10	8	8	8	10	9	6	6	5	5	2	1	3		
ID-1	11	11	11	11	11	11	10	9	9	8	4	5	6	2	5	
LDHB	≥11	≥11	≥8	≥8	≥8	≥8	≥9	≥10	≥10	≥8	≥11	≥12	≥11	≥14	≥12	≥14

The data in Table 2 were collated for 22 hybrid clones segregating mouse chromosomes that arose as independent mating events or exhibited differences in their isozyme patterns. The number of cases of lack of correlation (asyteny = +, −, or −, +) between two different isozymes was tabulated. In the case of LDHB (LDH1) where mouse and human phenotypes cannot be distinguished but VA2 has low LDH1 levels only + + + or + + activity was used as positive, and (−) used as negative. Mouse isozyme nomenclature is used in this table.

chromosome 2, and LDH B and PEP B with chromosome 12 have been reported¹³. VA2-mouse nervous system hybrids have yielded examples of asyteny of expression in the mouse for each of these isozyme pairs. Thus our studies suggest interspecies differences in these linkage relationships. We anticipate, however, that interspecies homology may be detected when markers short map distances apart or on the X chromosome are considered.

Jami *et al.*¹⁸ presented preliminary evidence of a case of retention of human and loss of mouse chromosomes in one and possibly two rare hybrids formed by fusing VA2 to mouse tissue culture L cells. In their report, however, no data were presented (such as absence of a mouse isozyme) which would imply loss of both homologues of any of the mouse chromosomes. Most of their VA2×L-cell hybrids, as well as similar hybrids produced by Weiss *et al.*² retained mouse and lost human chromosomes. Our results describe a general method (fusion of freshly liberated mouse cells with an established line of human cells) for producing reverse segregant hybrids as well as evidence that such strains do lose mouse chromosomes and isozymes.

The isozymes we studied were codominantly expressed in at least some hybrids from these matings and in hybrids of the type that retain mouse and lose human chromosomes¹³. It is likely for most isozymes studied that chromosome segregation, and not gene regulation, is responsible for the lack of expression. In addition, molecular hybridisation studies have demonstrated that mouse nuclear DNA can be lost altogether from such hybrids⁴. When mouse isozymes capable of codominant expression are not detected in hybrid cells, presumably both chromosome homologues have been lost from the cell line. Miller¹⁹ has shown in man-mouse hybrids segregating human chromosomes, however, that considerable heterogeneity of human chromosome complements can exist within a hybrid line in spite of a constant mean number of human chromosomes per cell. Thus, the total number of different chromosomes represented within a line may be quite large. Similar analysis will be needed of hybrids segregating mouse chromosomes.

The basis for the novel pattern of chromosome and isozyme segregation in these hybrids is undetermined. It does not appear to be a unique property of VA2 since similar results were obtained with human cell D98AH-2 (American Type Culture Collection CCL18.3). Nor are embryonic cells unique; adult mouse bone marrow and spleen cells also give reverse segregating hybrids with VA2. The nervous system cell fused (for example neurone or glia) is not known; however, further study of differentiated functions in the hybrids may establish their parentage. Since these hybrid strains retain complete human chromosome complements, they represent new combinations of human and mouse genetic information for work such as analysis of mitochondrial DNA composition⁴ or study of viral gene regulation²⁰.

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Activation of low molecular weight fragment of antihemophilic factor (factor VIII) by thrombin

THE precise molecular function of plasma antihemophilic factor (AHF, factor VIII), a glycoprotein with an apparent molecular weight of 2×10^6 , is unknown. Recent experiments suggest that this function may be carried out by a low molecular weight (LMW) fragment which can be dissociated from plasma AHF by increased salt concentration¹⁻³. The LMW fragment retains procoagulant activity, but is not reactive in immunoprecipitation assays for AHF-related antigen⁴. A high molecular weight (HMW) fragment, also identified using high salt conditions, has no procoagulant activity but retains AHF-related antigen in immunoprecipitation assays and von Willibrand factor activity in washed platelet assays^{4,5}. We have now found that the LMW fragment, as well as AHF⁶⁻⁹, is susceptible to activation by thrombin. This observation suggests that the LMW fragment is not a product of thrombin activation of AHF and verifies the proposed role of the LMW fragment in AHF procoagulant activity.

Partially purified AHF was obtained by agarose gel filtration of an AHF concentrate (Abbott Laboratories, South Pasadena) using the method of van Mourik and Mochtar¹⁰. AHF in the void volume fractions was concentrated by adding an equal volume of 30% polyethylene glycol (PEG); the precipitate was separated by centrifugation at 39,000g at 20° C for 30 min and was redissolved in one-tenth the original volume in imidazole

Table 1 Thrombin activation of AHF and LMW fragments

Incubation time (min)	AHF activity (U ml ⁻¹)	
	Purified AHF*	LMW fragments*
0	1.0	1.0
3	11	9.0
8	2.2	6.4
15	0.9	3.5
30	0.6	2.5
70	0.3	1.6

* Material was diluted in barbital-buffered saline⁴ so that each contained 1 U ml⁻¹ AHF activity. To each was added 1/20 volume phospholipid (final concentration 0.05 mg ml⁻¹) and 1/20 volume purified thrombin (final concentration 0.035 U ml⁻¹). The mixture was incubated for 3 min at 37° C and then placed in an ice water bath. Samples were removed for AHF assays¹² at the specified times. AHF activity before addition of thrombin is identified as 0 min. Control assays were carried out in which thrombin and phospholipid were added to buffered saline. This mixture had an apparent AHF activity less than 0.001 U ml⁻¹ and was not different from the blank time.

Table 2 Thrombin activation of AHF and HMW fragments

Incubation time (min)	AHF activity (U ml ⁻¹)	
	Purified AHF*	HMW fragments*
0	0.2	0.001
3	1.6	0.02
8	0.3	0.001
15	0.2	0.001

* Material diluted in barbital-buffered saline⁴ so that each contained 1 U ml⁻¹ of AHF-related antigen by radioimmunoassay¹³. Assay procedure was otherwise as described for Table 1.

(0.05 M)-buffered saline (0.14 M) pH 6.8. This sample was dissociated by gel filtration in 0.24 M CaCl₂ and 0.05 M imidazole, pH 6.8, using a Bio-Gel A-15m (BioRad) column of 1.6 × 30 cm with a flow rate of 20 ml h⁻¹ at room temperature⁴. HMW and LMW fractions were pooled separately and concentrated with an equal volume of 30% PEG as indicated above; the precipitates were each dissolved in one-tenth the original pool volumes in imidazole-buffered saline. Bovine thrombin (Parke-Davis) was purified by Lundblad's method¹¹. The specific activity of the thrombin was 1,333 U mg⁻¹ as determined by a reference curve prepared with standard thrombin supplied by Dr David Aronson (Bureau of Biologics Standards, Bethesda).

Thrombin activation of AHF and the LMW and HMW fragments was accomplished as described in Table 1. Activation of AHF and LMW fragment was maximal after 3 min, the procoagulant activity increasing tenfold over the starting activity (Table 1). Subsequent inactivation of procoagulant activity was less marked for the LMW fragment than for purified AHF. Results were similar in three experiments.

Low levels of AHF procoagulant activity were also detected 3 min after thrombin activation of the HMW fragment. Table 2 presents results from a typical experiment; shortening of the clotting times was observed in five instances. Thrombin activation of the HMW fragment may indicate incomplete dissociation of the LMW from the HMW fragment. Alternatively, masked potential procoagulant activity may be exposed by alteration of the inactive HMW fragment by thrombin.

Thus AHF fragments produced by dissociation in high ionic strength buffers retain the capacity for thrombin activation and inactivation. The demonstration that thrombin activates the LMW fragment provides additional evidence that this part of the AHF complex is important for AHF procoagulant activity as measured *in vitro*. A corresponding physiological role for LMW AHF fragments is suggested by the detection of low molecular weight procoagulant activity after transfusion in patients with von Willebrand's disease¹⁴.

The molecular basis for thrombin activation of AHF—and the LMW fragment derived from AHF—is unknown. As the LMW fragment retains a capacity for thrombin activation, it is unlikely to be a product of thrombin action on intact AHF. The absence of detectable proteolytic products when highly purified AHF is activated by thrombin^{15,16} supports this conclusion. Thrombin activation of AHF is an important but poorly understood phenomenon; studies with LMW fragments will permit more probing analysis of this interaction.

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Myosin linked calcium regulation in vertebrate smooth muscle

CONTRACTILITY resulting from the interaction of actin and myosin filaments is in general controlled by the concentration of calcium ions. The regulation of this interaction by Ca ions can, in principle, be exercised at the level either of the actin or of the myosin filaments and both these types of regulatory system are found in nature. In vertebrate skeletal muscle troponin is the Ca receptor and is attached to the actin filament. The action of troponin is inhibitory; when no Ca ions are bound to it the interaction between actin and myosin is prevented^{1–4}. This inhibition is relieved and interaction occurs at calcium concentrations sufficient for the Ca binding sites on the molecule to be occupied. The second type of regulatory system was discovered in molluscan muscles and is present in other invertebrate systems which lack troponin^{5,6}. In some cases both types of regulation seem to be present in the same muscle^{6,7}. Here I present evidence which shows that such a myosin-linked regulatory system is not confined to invertebrates but is also present in smooth muscles of higher vertebrates. A more detailed description of the regulatory system in this muscle type is presented elsewhere^{8,9}.

Rabbit actin filaments without regulatory proteins attached have been used in a diagnostic test for the presence of myosin-linked Ca regulatory systems⁶. The principle underlying this competitive actin-binding assay is that if an actomyosin contains Ca-regulated myosin filaments, the ATPase activity of the preparation will not be stimulated when unregulated actin filaments are added; Ca ions will still be required for activation. This is the case when purified rabbit actin filaments¹⁰ are added to smooth muscle (chicken gizzard) actomyosin (Table 1). The ATPase activity shows the identical sensitivity to Ca both in the presence and absence of a molar excess of actin molecules. Other experiments have established that actin and myosin of smooth muscle actomyosin dissociate under the conditions used in this experiment and thus the myosin molecules were free to complex with the added skeletal muscle actin. The turbid smooth muscle actomyosin undergoes a dramatic clearing reaction when ATP is added, coincident with the dissociation of actin and myosin. Observation of a reaction mixture indicated that no incipient precipitation occurred when the actin filaments were added and therefore that the actomyosin remained dissociated. Since Ca is required for stimulation of the ATPase activity in the presence of unregulated skeletal actin, the absence of Ca must have a direct effect on the myosin which prevents its interaction with actin. Precisely how Ca determines whether or not the myosin will combine

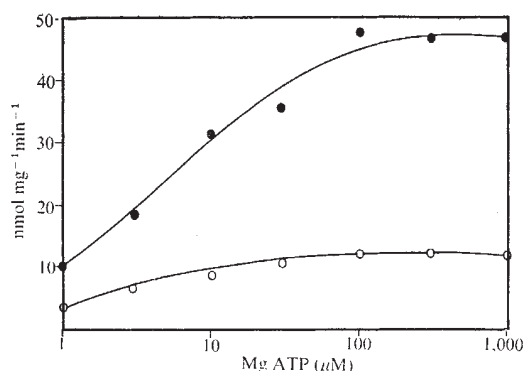


Fig. 1 The rate of ATPase activity was determined using a creatine kinase ATP regenerating system⁸. The reaction medium contained 60 mM KCl, 20 mM imidazole (pH 7.0), 5 mM creatine phosphate, 0.5 mg ml⁻¹ creatine kinase and 5.3 mg ml⁻¹ GAM, 1 mM MgCl₂, 0.5 mM cysteine, and either 2 mM EGTA or 100 μM CaCl₂. The MgATP concentration indicated is the added concentration, the myosin concentration is about 7.3 μM. ATP was added as the Mg chelate, and therefore the Mg²⁺ concentration, was 1 mM throughout the experiment. The reaction time varied between 3 and 30 min at 25°C. ●, +Ca; ○, -Ca.

with actin is not understood; it could be through an electrostatic effect or through a conformational change in the molecule.

In vertebrate smooth muscle preparations, no troponin-like components can be identified by SDS gel electrophoresis^{8,9}. But to demonstrate the absence of troponin by another, independent method, a functional test, which is essentially the converse of the experiment above, was carried out. For this experiment rabbit heavy meromyosin (HMM) which itself is not calcium responsive was mixed with the smooth muscle actomyosin (Table 1). If no Ca regulatory system is present on the actin filaments the HMM ATPase activity would be expected to be unrestrained at low Ca concentrations; otherwise it would be inhibited. As expected of an actomyosin without Ca-regulated actin filaments, the ATPase activity after HMM addition is less sensitive to Ca than the control.

Subtracting the contribution of the smooth muscle actomyosin from the total activity shows that the actin stimulation of the HMM ATPase activity is actually depressed in 100 μM Ca. Since the smooth muscle actomyosin superprecipitates in the presence of 100 μM Ca, this depression can be accounted for by the complexing of the smooth muscle

myosin with a fraction of the actin molecules, thereby reducing the number available to stimulate the HMM ATPase activity.

Another distinguishing feature of actin-linked compared with myosin-linked regulatory systems is their response to changes in MgATP concentrations¹¹. Relaxation of skeletal muscle systems does not occur at low MgATP concentrations where the myosin active sites are not saturated with substrate. The raised ATPase activity^{3,4,12} and persistence of contraction¹³ which occur under these conditions is indirectly caused by the substrate-free myosin molecules. They form rigorous complexes with actin and nullify the inhibitory influence of Ca-free troponin^{3,4}. If myosin is subject to Ca regulation, such a biphasic response—stimulation at low and inhibition at high MgATP concentrations—is not expected and is not found in myosin-linked regulatory systems¹¹.

Smooth muscle actomyosin responds in a manner typical of a myosin-linked regulatory system; the response is not biphasic. ATPase activity at the low Ca concentration increases monotonically, and is Ca sensitive (that is, relaxation occurs, at all MgATP concentrations even when the number of myosin molecules exceeds that of substrate molecules) (Fig. 1). Similar results have been obtained in superprecipitation experiments with actomyosin and in ATPase activity measurements and superprecipitation experiments with smooth muscle myofibrils.

My results are consonant with those of Filo *et al.*¹⁴ where tension measurements were performed at different MgATP concentrations on guinea pig taenia coli. In their study, however, the concentration of MgATP was changed by adding various amounts of Mg to a constant amount of ATP. The interpretation of the data obtained under these conditions is not straightforward because the ATP anion has a relaxing effect on smooth muscle^{8,9,15}, and also because Mg ions have specific effects on smooth muscle contractile apparatus^{8,9}. The results of these workers indicate nevertheless, that myosin regulation is not restricted to chicken gizzards.

The above results indicate that the myosin-linked calcium regulation found in primitive organisms has been retained during the evolution of higher vertebrates. Both types of regulatory system have evolved in parallel and are present in different types of muscle in the same animal. In view of the similarity between the myosin of smooth muscle and that from non-muscle sources, especially with respect to their immunological characteristics^{16,17}, and with respect to the light chain size^{8,9,18-21} it is not unlikely that a similar mode of Ca regulation is present in these other systems as well. If this can be substantiated, then the study of smooth muscle, which is more readily available, will throw light on these other less well defined forms of contractility.

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Table 1 Locus of the calcium regulatory system in smooth muscle actomyosin

Components	ATPase activity nmol ml ⁻¹ min ⁻¹		Ca sensitivity (Activity 10 ⁻⁴ M Ca) (Activity 10 ⁻⁶ M Ca)
	10 ⁻⁶ M Ca ²⁺	10 ⁻⁴ M Ca ²⁺	
(a) Gizzard actomyosin (GAM) (1.3 mg ml ⁻¹)	4.1	21.7	5.3
(b) GAM (1.3 mg ml ⁻¹) + rabbit actin (1 mg ml ⁻¹)	4.4	23.2	5.3
(c) GAM (1.3 mg ml ⁻¹) + rabbit actin (1 mg ml ⁻¹) + chicken breast tropomyosin (0.2 mg ml ⁻¹)	4.4	23.1	5.3
(d) GAM (1 mg ml ⁻¹) + Rabbit HMM (0.5 mg ml ⁻¹)	29.1	39.7	1.4
(e) (d) - (a)	25.0	18.0	0.7

The weight ratio of tropomyosin:actin:myosin in the smooth muscle actomyosin is approximately 0.3:1.0:2.4 (ref. 8). The ATPase activity was determined by phosphate liberation after mixing gently for 10 min at 25°C in a medium containing 1 mM MgATP, 40 mM KCl, 20 mM imidazole (pH 7.0), and either 1 mM EGTA or 100 μM CaCl₂. The rabbit actin filaments and the chicken breast tropomyosin were determined to be free of contaminating proteins by SDS gel electrophoresis analysis. In (D) the rate of HMM ATP hydrolysis in the absence of actin (3.4 nmol ml⁻¹ min⁻¹) has been subtracted and thus the values given are actin-stimulated activities.

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Calcium ionophore A23187 potentiates twitch and intracellular calcium release in single muscle fibres

THE potentiation by drugs of the twitch force in skeletal muscle has generally been related to a prolongation of the 'active state' in the excitation-contraction coupling mechanisms^{1,2}. This can, however, only be partially correct since in another type of potentiation elicited by repetitive stimulation (staircase phenomenon), the 'active state' was found to be intensified but not prolonged³. This view that the 'active state' is not maximum in a single twitch and that it can indeed be intensified in twitch potentiation was criticised⁴ but subsequently received support from several studies^{5,6}. On the other hand it is well known that activation of muscle contraction involves rapid changes of the intracellular calcium concentration which result from calcium release and uptake processes in the sarcoplasmic reticulum (SR)^{7,8}. The discovery of specific ionophores (antibiotics allowing cations to cross biological membranes by way of a fairly electroneutral mechanism) which selectively increase the membrane permeability to calcium^{9–11} offers a new tool for probing these intramuscular processes. For example, ionophore X537A was found to alter the contractile responses and resting tension of strips of rat diaphragm, an effect which was ascribed primarily to an increased calcium influx across the muscle fibre membrane¹². On the other hand the ionophores X537A and A23187 tested on SR vesicles and mitochondria isolated by ultracentrifugation were found to induce a release of stored calcium^{10,12,13}.

The present investigations indicate that the calcium ionophore A23187 (Eli Lilly) strongly potentiates the twitch force of single muscle fibres and acts directly on the process of calcium release from the SR, even in these intact fibres. The experiments were performed on muscle fibres isolated from the depressor muscles of the barnacle, *Balanus nubilus*, in conjunction with two different procedures as described recently¹⁴: (a) the intracellular concentration of calcium ions at rest and during activation was studied in large fibres 1.0 to 2.0 mm diameter which had been micro-injected with the calcium-sensitive bioluminescent protein aequorin¹⁵ and placed in a light-tight chamber in front of an EMI 9635 photomultiplier tube; (b) the calcium outflux across the outer membrane was estimated in single barnacle fibres loaded by an intracellular injection of 0.1 to 0.2 μ l ^{45}Ca in Tris buffer at pH 7.2 (ref. 16). The calcium ionophore A23187 was dissolved at 10^{-3} M in ethanol

which was added to artificial seawater (ASW) to obtain a final concentration chosen from 10^{-7} to 10^{-5} M.

Figure 1 illustrates the resting calcium outflux from a single barnacle muscle fibre equilibrated in ASW, two hours after ^{45}Ca loading. Ethanol 1% added to the external medium was found to increase the resting outflux by 90% above baseline. At these concentrations the ethanol effect reached its maximum in about 30 min. After this control test A23187 10^{-5} M in ASW (thus containing also 1% ethanol) was substituted externally: the resting calcium outflux increased markedly to reach a new level, 450% above the extrapolated baseline. The maximum increase of calcium outflux induced by A23187 was usually observed after 30 to 50 min in 10^{-5} M A23187. The increase of ^{45}Ca outflux was recorded in all experiments but its amplitude depended upon the ionophore concentration; the recovery on return to ASW was influenced both by the duration of exposure and by the concentration used. The findings cannot be explained by an increased exchange diffusion across the outer fibre membrane because similar A23187-induced augmentations of ^{45}Ca outflux were recorded in experiments with 0 Ca in the external solution. These data suggest that the intracellular calcium storage sites are involved.

This was tested by recording the rate of light emission of a barnacle fibre micro-injected with aequorin^{15,14}. The fibre was stimulated at intervals of more than 30 s through an intracellular silver wire with square electrical pulses of 75 or 150 ms duration of various intensities. The membrane potential was monitored with another intracellular platinum electrode connected to a

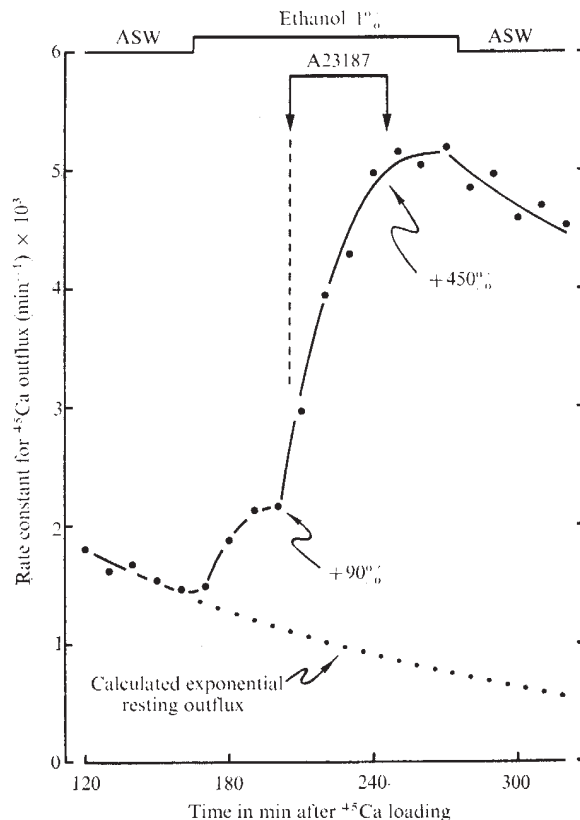


Fig. 1 Effect of A23187 10^{-5} M on the ^{45}Ca outflux from a single barnacle muscle fibre. The exponential resting outflux curve ($y = ae^{bx}$; $a = 57.09$ and $b = -0.01$; coefficient of determination $r^2 = 0.7831$) was calculated from the experimental data recorded after 2 h equilibration of the fibre in ASW. The ionophore solvent, ethanol, modified the resting outflux: 90% above baseline after 30 min. This was followed by a marked increase of the outflux (450% in 40 min) when A23187 was added to the external medium. Only a moderate recovery was observed when this fibre was transferred back to ASW. Fibre diameter: 1.0 mm; mean intracellular resting potential: -48 mV; temperature of solutions: 22°C .

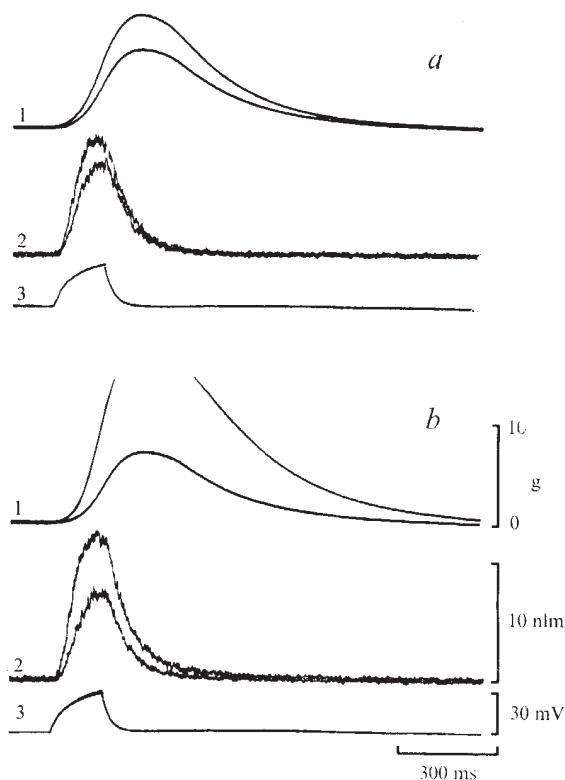


Fig. 2 Result of applying a single electrical stimulation of 150 ms duration to a barnacle muscle fibre injected with aequorin. Two traces are superimposed in each record: one before the application of A23187 serving as control and the other after the ionophore had been present for 10 min. Trace 1, isometric tension; trace 2, calcium mediated light emission; trace 3, membrane response. The slight decrease observed in trace b,3 was recorded in presence of A23187. Fibre diameter: 1.9 mm; mean intracellular resting potential: -54 mV; temperature of solutions: 23°C . a, A23187 (10^{-6} M); b, A23187 (10^{-5} M).

high input impedance amplifier^{15,14}. Figure 2 illustrates typical results obtained on the same muscle fibre in the presence of two different concentrations of A23187, under identical stimulation conditions. Two traces are superimposed in each record, one before the application of A23187 serving as control and the other after the calcium ionophore had been present in the external medium for 10 min.

The membrane depolarisations actually elicited by the constant intensity stimulus (record 3) were stable in the control and test trials which indicates that any difference in the force output (record 1) or in the calcium transient (record 2) must result from genuine changes in the mechano-chemical activation processes (compare ref. 15). No regenerative action potential was elicited by the stimuli as shown by the intracellular record 3. In the presence of 10^{-6} M A23187 the mechanical force output (record 1) increased by 42% but it was not prolonged (Fig. 2a). The size of the calcium transient (record 2) increased by 28% in Fig. 2a and its rate of rise was much accelerated so that the peak was reached somewhat earlier. The duration of the calcium transient was not significantly prolonged and the time from peak to half decay was indeed slightly reduced. A concentration of 10^{-5} M A23187 was tested in Fig. 2b. After 10 min the force was considerably increased to about 3 times control. The rate of force development was accelerated. The calcium transient was increased by 70%, its rate of rise was accelerated and the time from peak to half decay was about the same as that of control. These results were regularly obtained in 5 muscle fibres from 4 different barnacles, each one being tested with different combinations of stimulus parameters and of ionophore concentration from 10^{-7} to 10^{-5} M. The above modifications returned to

control values when the fibre was transferred to ASW without ionophore in a time variable from 10 to 20 min.

The above results suggest that when the calcium ionophore A23187 10^{-7} to 10^{-5} M is added to the fluid bathing a single giant muscle fibre, its action involves not only the outer membrane (Fig. 1) but also the intracellular calcium storage sites. The latter effect was very strong and a standard depolarisation step imposed to the outer membrane evoked a larger and faster release of calcium which in turn elicited a much potentiated twitch (Fig. 2). The resting light emission was only slightly increased when the potentiation was recorded. The A23187 potentiation which occurs without any significant prolongation of the mechano-chemical events presents a formal resemblance to the staircase potentiation³ which we consider to involve primarily the calcium release from SR. The effect of A23187 has a rather rapid onset and large potentiations of the contraction can be recorded after only 1 or 2 min of exposure to A23187. The effect is reversible and does not apparently disturb the sequence of events involving calcium sequestration by SR during the relaxation of the muscle fibre. The records of Fig. 2 make it clear that the calcium ionophore at these concentrations provides a useful tool to manipulate intracellular processes of excitation-contraction coupling in skeletal muscle fibres directly.

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Precocious development of detoxicating enzymes following pituitary graft

IN vertebrates many biologically-active endogenous compounds, (such as bilirubin, steroid hormones and the catecholamines) and an enormous range of drugs, carcinogens, pesticides, industrial pollutants or their metabolites are made more polar and excreted by conjugation with glucuronic acid¹. This is the principal route of detoxication but only one mechanism for the biosynthesis of such 'glucuronides' is known:

Table 1 Effect on embryo-liver UDP glucuronyltransferase activity of grafting various adult tissues on to chorioallantoic membrane

Tissue	nmol <i>o</i> -aminophenyl glucuronide formed per mg protein h ⁻¹
None (sham-operated)	0.6, 0
Adrenal gland	0, 0
Testis	0, 0
Skeletal muscle	0.6, 0
Liver	1.0, 0
Brain	1.1, 0.5
Pituitary gland	6.5, 6.3, 6.5

Tissue from 6 week White Leghorn cockerel was placed on the chorioallantoic membrane of 12 d embryos, and GT activity assayed¹ in embryo livers on day 15. Results are from separate experiments. Weights of the other tissues were 1–3 times the weight of the single pituitary gland grafted.

glucuronyl transfer by UDP glucuronyltransferase (GT) (EC 2.4.1.17.) to the accepting compound¹.

GT occurs only at very low levels in the embryo or foetus¹; it reaches adult levels around birth, the rate depending on substrate and species. Because much drug toxicity and jaundice in the newborn arises from this temporarily defective GT activity, the search for factors controlling induction of the enzyme has been extensive.

GT activity can be increased in newborn² or term-foetal liver (B. Burchell and G. J. D., unpublished) by phenobarbital, and this drug can induce GT in chick embryo liver *in ovo*³ or in culture⁴. The endogenous inducers or inducing mechanisms, however, have remained unknown.

We report here the precocious increase of GT from virtually zero to adult levels in the livers of chick embryos *in ovo*, following the grafting of chicken pituitary gland on to the chorioallantoic membrane. This observation should enable the identification of the endogenous inducing mechanism of this major detoxicating enzyme. We have found that pituitary grafts also allow prematurely adult expression of one of the microsomal mixed-function oxidases (EC 1.14.1.1.), enzymes which hydroxylate a molecule before glucuronidation⁵ and which are therefore also important in today's environment.

GT activity is detectable in chick embryo liver at 8 d but remains very low until hatching at 21 d, when it increases dramatically to adult levels⁶. Because the culture of isolated liver out of the embryonic environment allows precocious induction of GT⁷, an *in ovo* 'repression' of the enzyme has been postulated. We were unable to overcome this repression by varying O₂ and CO₂ tensions, by incubating embryo and membranes in an open dish, by hypophysectomising the embryo at 36 h (ref. 8) or by injecting known hormones from mammalian adrenal cortex, thyroid and pancreas.

The pituitary begins to secrete in foetuses just before the emergence of the late-foetal cluster of enzymes⁹, amongst which is GT; and an injection of pituitary extract brought about the precocious appearance of xanthine oxidase in chick embryo liver¹⁰. We therefore attempted to evoke GT by injecting various commercial extracts or freshly-made homogenates of mammalian or chicken pituitary glands into the egg. Our results were inconclusive, and we decided to graft adult chicken

glands on to the chorioallantoic membrane to allow a more physiological uptake of possibly unknown factors.

Pituitaries were therefore taken from 6–8 week old hens or cockerels and grafted, one per egg, on to the chorioallantoic membrane of 12 d eggs. Other eggs received grafts of other tissues, control eggs were sham operated and all eggs were then returned to incubation. Embryo livers were examined on subsequent days. By the third day, embryo-liver GT activity had risen markedly in those eggs which had received grafts of pituitary gland; adult brain, adrenal, testis, liver and muscle grafted on to the membrane did not significantly alter the normal negligible embryo-liver levels of the enzyme (Table 1).

The rise in GT activity was time-dependent. It was not notable until day 3 after grafting and increased during days 3 and 4. It reached the adult range on day 4 (Table 2).

Activity of the mixed-function oxidase, aniline hydroxylase¹¹, was also increased by pituitary-grafting; it rose threefold, reaching adult level. No rise was observed for either enzyme in embryo livers under 10 d old at the time of grafting and examined up to 4 d later.

Table 3 Age of pituitary gland donors and effect on embryo-liver UDP glucuronyltransferase

Donor	nmol <i>o</i> -aminophenyl glucuronide formed per mg protein h ⁻¹
No donor (control)	0.6 ± 0.2 (5)
15 d embryo	0.9 ± 0.3 (5); 18.2*
18 d embryo	1.8 ± 0.6 (5); 11.2*
20 d embryo (not hatching)	1.8
20 d embryo (hatching)	26.5, 26.4
1 d chick	1.5, 4.8, 6.3
2 d chick	12.9
6 week cockerel	32.9 ± 15.1 (7)

One pituitary gland from the donor was placed on the chorioallantoic membrane of a 12 d embryo, and liver GT assayed¹ on day 16. Results expressed as in Table 2.

*Two glands placed on membrane.

Pituitaries from 2 d-old chicks proved almost as effective as those from adults. Younger pituitaries, which were of approximately similar weight from 2 d chicks down to 8 d embryos, appeared progressively less effective except from those birds actively hatching (Table 3). Doubling the amount of embryo gland grafted evoked a marked response, however (Table 3), suggesting a threshold phenomenon.

The stimulatory effect is produced by the cephalic region of the anterior pituitary; the caudal region has negligible effect when grafted. Partly-purified preparations of hormones found in the cephalic region¹² proved ineffective when applied to the membrane at various time intervals. Pituitaries from mouse and rat have so far also failed to increase embryo-liver GT, although the grafts were apparently accepted.

The cephalic region of the chick anterior pituitary gland therefore secretes a factor or factors which in the intact embryo *in ovo* causes precocious development of hepatic GT and aniline hydroxylase. Embryos over 9 d old are competent to respond to this factor, production of which seems enhanced by the process of hatching. The nature of the factor is now being determined and its role in the natural development of these detoxicating enzymes is being investigated.

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Table 2 Development of UDP glucuronyltransferase activity in liver of embryos exposed to pituitary grafts

Day	nmol <i>o</i> -aminophenyl glucuronide formed per mg protein h ⁻¹
	Test Control
14	0.7, 0.5, 1.1, 1.3 0.6 ± 0.4 (5)
15	6.5, 6.3, 6.5 0.6 ± 0.4 (5)
16	32.9 ± 15.1 (7) 0.6 ± 0.2 (5)

Pituitary gland from a 6 week cockerel was placed on chorioallantoic membrane of 12 d embryo and GT assayed¹ in embryo liver on days noted. Results are of individual experiments or s.e.m. with number of experiments in parentheses. 'Adult range' for this batch of 6 week cockerels was 30–60 of above units.

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Transcription, translation and maturation of succinate dehydrogenase during cell cycle

A STEPWISE accumulation of individual enzyme activities is a common feature of the eukaryotic cell cycle¹. Two major hypotheses consider that the temporal restriction is produced by oscillatory repression or by linear reading of genes along the chromosome^{2,3}. Both hypotheses, in their present form, assume that increase in enzyme activity is concurrent with transcription and translation. We have made use of the high degree of synchrony obtainable with *Chlorella fusca* var. *vacuolata* 211-8p, grown under intermittent illumination^{4,5}, to study the duration of transcription and translation for succinate dehydrogenase by applying inhibitors during the period of its stepwise increase in activity. We report that the periods of transcription, of translation and of increase in activity are only partially concurrent.

Cycloheximide specifically inhibits protein synthesis of this strain of *Chlorella*⁶ and its addition at 15 h in the 24 h cycle completely prevents the stepwise accumulation of succinate dehydrogenase (Fig. 1a) which would normally begin then⁷. It is striking that addition of the inhibitor less than 0.1 cycles later, at 17 h, when only a third of the full increase in activity has occurred, is unable to prevent the full subsequent accumulation of enzyme activity. Measurement of phenylalanine incorporation into protein, however, shows that cycloheximide retains its ability to inhibit protein synthesis even when the increase in succinate dehydrogenase activity is immune to its presence (Fig. 1b). The inhibition of protein synthesis is not an artefact caused by loss of labelled amino acid from the cells, as whole-cell counts show retention of the pool during exposure to cycloheximide (Fig. 1c). Therefore the accumulation of succinate dehydrogenase activity rapidly enters a phase in which it is not dependent upon continued protein synthesis by the cytoplasmic ribosomes. As succinate dehydrogenase is an enzyme of the inner mitochondrial membrane^{8,11}, however, and as much of this membrane is synthesised within the organelle^{11,12}, we investigated the possibility that the period when enzyme activity accumulates without cytoplasmic protein synthesis is one in which proteins must be synthesised by the mitochondrial ribosomes to allow the enzyme to develop into its catalytically active form. Chloramphenicol was shown to be an effective inhibitor of mitochondrial protein synthesis during this period of the cycle because it prevented the accumulation of cytochrome oxidase (Fig. 2a), an enzyme known from very diverse sources to contain some proteins of mitochondrial origin^{12–14}. The presence of chloramphenicol from as early as 11 h was, however, not at all inhibitory to the accumulation of succinate dehydrogenase (Fig. 2b). Similarly, lincomycin at 0.2 mg ml⁻¹ inhibited cytochrome oxidase but not succinate dehydrogenase synthesis. Therefore that period of the increase in enzyme activity after 17 h, which does not require con-

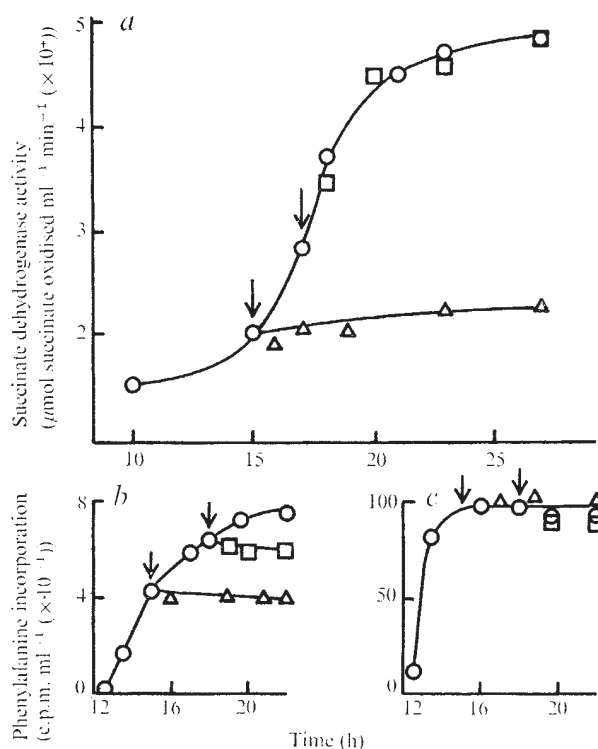


Fig. 1 The effect of cycloheximide, *a*, on the accumulation of succinate dehydrogenase activity, *b*, the incorporation of phenylalanine into protein and, *c*, the uptake of phenylalanine. Cells were synchronised and held at a density of 5×10^6 ml⁻¹ under a regime of 15 h light and 9 h dark⁴ which was maintained during the experimental period. Cycloheximide (Δ , $\square$) was applied at 2.5 μ g ml⁻¹ (arrows), and ¹⁴C-L-3-phenylalanine (U) was added at 10^{-3} M and 25 μ Ci mmol⁻¹. The culture was sampled and assayed for succinate dehydrogenase activity as described previously⁷ with the modification that for 10 min of the 15 min *in vitro* succinate activation⁸ period, the enzyme was incubated with 0.15 M succinate, pH 7.4, before addition of the assay reagents. For estimation of phenylalanine incorporation into protein, the following procedure was necessary to completely leach free phenylalanine through the sporopollenin wall^{9,10}. Five ml of culture was mixed with 15 ml of ethanol and stored at -15°C. The cells were pelleted, washed in cold growth medium containing 5×10^{-3} M cold phenylalanine and extracted with ethanol at 80°C for 3 min and ether at 60°C for 1 min. The protein was dissolved in 0.2 ml of 8 M urea containing 5×10^{-3} M phenylalanine and 15 mg ml⁻¹ bovine serum albumin and was then precipitated with cold 5% trichloroacetic acid, washed with cold water, extracted in 0.5 M H₂SO₄ at 100°C for 3 h, washed briefly with hot water and dissolved in 0.1 ml of 10 M NaOH. For counting, the alkali was neutralised with 0.3 ml of 1 M phosphate buffer, pH 6.8, and 0.1 ml of 10 M HCl, and 0.2 ml samples were mixed with 0.8 ml H₂O and 10 ml scintillant, which contained 50 ml Triton X-100 and 0.7 g butyl-PBD per 100 ml toluene. For measurement of phenylalanine uptake, 5 ml samples of culture were passed through a membrane filter and the cells washed three times with cold growth medium then dried overnight at 105°C and counted with 3 ml of scintillant. $\circ$, Control curve.

current cytoplasmic protein synthesis (Fig. 1a), does not depend on mitochondrial protein synthesis either.

The increase in activity cannot be attributed to a change in the substrate affinity of the enzyme, for we shall show elsewhere that the K_m values for succinate and phenazine methosulphate do not change significantly. Neither does the accessibility of the enzyme in cell extracts vary, because the mitochondrion forms a single reticulum¹⁵ which is always completely disrupted by the force necessary to break the sporopollenin-containing cell wall⁹. Instead there seems to be a real increase in the number of catalytically active enzyme molecules between 17 and 24 h, due to the maturation of enzyme protein made between 15 and 17 h.

To investigate the relationship between transcription and the

2 h period of translation we have employed 6-methylpurine. This analogue inhibits RNA synthesis both in higher plants¹⁸⁻¹⁹ and in this strain of *Chlorella*, where its effect is specifically to inhibit functional RNA synthesis by competition with adenine¹⁹. When 6-methylpurine is added at 12 h the subsequent synthesis of succinate dehydrogenase is completely inhibited (Fig. 3a). There is no evidence, therefore, of a persistent mRNA for the enzyme throughout the cell cycle. When added at 18 h, after translation has ceased, the inhibitor had no effect (not shown). There is, however, evidence that the newly transcribed mRNA is not immediately translated as, although translation does not begin until 15 h (Fig. 1a), the addition of 6-methylpurine to a different sample from the same culture at 15 h reveals that the inhibition of RNA synthesis is only able to inhibit half of the subsequent enzyme accumulation (Fig. 3a). Thus, about half of the necessary mRNA may have already been transcribed at the time of initiation of translation.

This conclusion is only valid if 6-methylpurine is able to cause immediate inhibition of functional RNA synthesis: therefore its ability to compete with adenosine for incorporation into RNA was investigated at this time in the cycle. Although the analogue competed with adenosine for uptake, there was no loss of labelled adenosine from the intracellular pool (Fig. 3b) but there was an immediate 65% displacement of adenosine from incorporation into RNA (Fig. 3c). Purified tritiated 6-methylpurine is itself incorporated into all sizes of RNA separated by gel electrophoresis (C. A. Lambe and P.C.L.J., unpublished).

The evidence obtained by use of 6-methylpurine therefore suggests the presence of some mRNA for succinate dehydrogenase before translation begins. Yet there is no evidence of mRNA before 12 h in the cycle (Fig. 3a) and so the maximum period during which mRNA may be present, before translation begins at 15 h, is less than 0.15 cycles. Therefore, the time of succinate dehydrogenase synthesis is closely determined by the time of transcription. This is the first time that transcription has been shown to have a role in determining the time of syn-

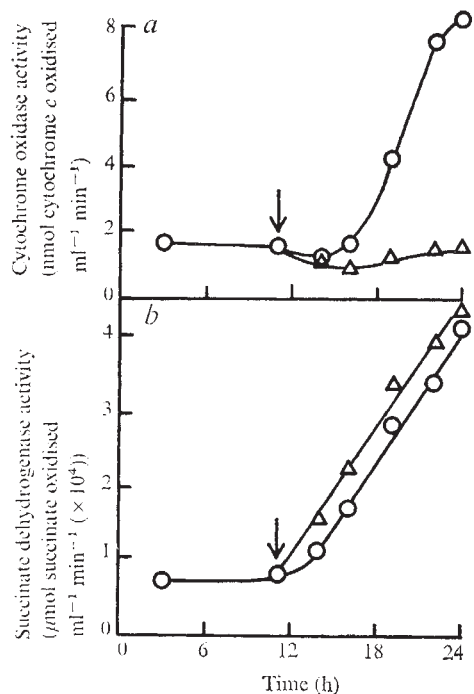


Fig. 2 The effect of chloramphenicol on, a, the synthesis of cytochrome oxidase and, b, succinate dehydrogenase. D-threo-chloramphenicol (Δ) was applied at 0.8 mg per ml at the times arrowed, and the cells were cultured as in Fig. 1. The culture was sampled and assayed for cytochrome oxidase as described previously⁷ and for succinate dehydrogenase as in Fig. 1. $\circ$, Control curve.

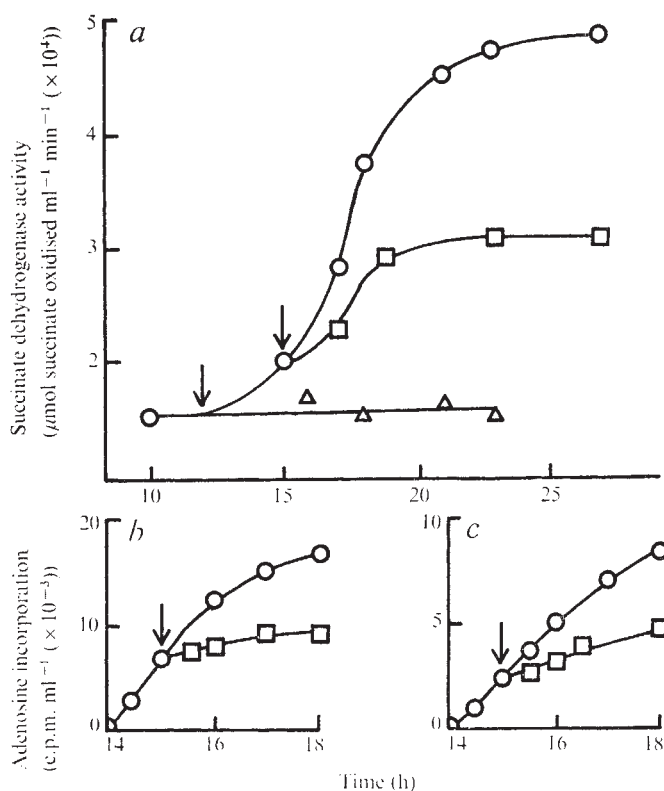


Fig. 3 The effect of 6-methylpurine on, a, the synthesis of succinate dehydrogenase, b, the uptake of adenosine and, c, the incorporation of adenosine into nucleic acid. The cells were grown as described in Fig. 1 and 6-methylpurine (Δ , $\square$) was added (arrows) at 1×10^{-3} M. ¹⁴C-adenosine (U) was supplied at 1.25×10^{-6} M and 26 mCi mmol⁻¹. For measurement of total cellular uptake, 5 ml of culture was filtered through a double thickness of Whatman GF/A glass fibre filter and washed twice with cold growth medium containing 1.25×10^{-6} M cold adenosine, then dried at 75°C overnight and covered with scintillant as in Fig. 1. For estimation of adenosine incorporation into nucleic acid, 5 ml samples of culture were made up to 5% perchloric acid and stored at 0°C. The cells were filtered, washed with 5% cold perchloric acid dried at 105°C and counted in the same way. $\circ$, Control curve.

thesis of a stepwise accumulated enzyme and there are few other investigations of the role of transcription in determining any pattern of synthesis in the cell cycle. In the thermophilic strain of *Chlorella* the glutamine analogue azaserine immediately inhibits synthesis of two autoregulated enzymes^{20,21} and 6-methylpurine prevents the increase in glucose-6-phosphate dehydrogenase activity which occurs in excised artichoke tissue¹⁸.

There is therefore some support for the conclusion that transcriptional regulation can govern the pattern of enzyme synthesis in the cell cycle, but the delay observed here before translation of the mRNA might indicate that post-transcriptional regulation also operates. Control at this level is believed to govern isocitrate lyase synthesis under catabolite repression²² and nitrate reductase synthesis under ammonia repression⁵ in this strain of *Chlorella*. Post-transcriptional controls may also be involved in the synthesis of tyrosine aminotransferase in hepatoma cells^{23,24} and in the synthesis of some proteins²⁵⁻²⁷ in other developmental situations analogous²⁸ to progress through the cell cycle. At least some of the pre-formed mRNA, however, will be mRNA undergoing the normal procedure of processing and export from the nucleus. We therefore believe that the period when mRNA for succinate dehydrogenase is available in the cytoplasm but remains untranslated, can only be brief in relation to the cell cycle.

The enzyme is not matured into a catalytically active form before incorporation into the mitochondrial membrane, as no significant activity can be detected in the soluble fraction,

after centrifugation for 30 min at 100,000g, at any time during the phase of increasing activity. This raises the possibility that the maturation process depends on the rate-limiting synthesis of new inner mitochondrial membrane to accommodate the enzyme. In fact, a burst of mitochondrial membrane synthesis at just this stage of the cycle, after S phase, is predicted by a recent hypothesis^{29,30}.

Stereology has revealed that there is a continuous increase in mitochondrial volume during the cycle^{5,15}, and furthermore we have found that the presence of chloramphenicol from 11 h in the cycle, which would be expected to block inner membrane synthesis^{11,12,14}, still allows the formation of fully particulate enzyme. Maturation is thus not limited by the availability of membrane to accommodate the new enzyme. Another factor, perhaps the rate of incorporation into the membrane or the addition of the flavin prosthetic group⁸, must determine the rate at which catalytic activity develops.

We conclude that the stepwise synthesis of succinate dehydrogenase is initiated by a brief period of transcription which begins perceptibly before the 2 h period of translation; however, enzyme activity accumulates for a total of 9 h because there is a further, rate-limiting, step in the formation of active, membrane-bound enzyme.

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Feedback control of vitamin D metabolism by a nuclear action of 1,25-dihydroxycholecalciferol on the kidney

1,25-DIHYDROXYCHOLECALCIFEROL (1,25 DHCC) is the most active metabolite of vitamin D₃^{1–4}, and its production by the kidney *in vivo* varies with the calcium and vitamin D content of the diet⁵. In spite of several theories, the mechanisms by which dietary changes cause alterations in 1,25 DHCC production are not completely understood. Hormonal^{6–8} and ionic^{9–11} messengers have been suggested, and both may have a role.

In a previous report¹⁰, we described inhibition of the conversion of 25-hydroxycholecalciferol (25 HCC) to 1,25 DHCC *in vitro* after incubation of isolated chick kidney tubules with 1,25 DHCC. We show here that this inhibition is not due to a direct competitive effect of 1,25 DHCC, but that it is probably due to an action on the nucleus. Because of a dependence of this effect on the calcium ion concentration, and by analogy with the mode of action of 1,25 DHCC in the intestine, it is proposed that 1,25 DHCC induces the formation of proteins involved in calcium movement into and across the cell. The known sensitivity of the 25 HCC-1-hydroxylase enzyme to calcium^{8,9} suggests that this enhanced calcium entry into the cell may explain the inhibitory effect of 1,25 DHCC on the 25 HCC-1-hydroxylase enzyme. The proposed mechanism would explain the interdependence of 25 HCC-1-hydroxylase activity, and the calcium and vitamin D nutritional state observed *in vivo*.

Table 1 Effect of preincubation with 1,25 DHCC (3.75 nmol l⁻¹ in (a) and 25 nmol l⁻¹ in (b)) for (a) different time intervals, and at (b) different calcium concentrations on the conversion of (26-(27)-methyl-³H)-25 DHCC to 1,25 DHCC by isolated renal tubules from vitamin D-deficient chicks

Experimental situation	1,25 DHCC	Recovered radioactivity (%) as 1,25 DHCC (Mean $\pm$ s.e.m.)	Significance
(a)			
10 min	—	30.6 $\pm$ 0.8	
	+	32.0 $\pm$ 1.2	n.s.*
30 min	—	32.8 $\pm$ 1.0	
	+	35.4 $\pm$ 1.0	n.s.
150 min	—	41.8 $\pm$ 5.1	
	+	21.3 $\pm$ 3.3	P < 0.02
(b)			
0.6 mmol l ⁻¹ Ca ²⁺	—	11.8 $\pm$ 0.3	
	+	11.6 $\pm$ 1.2	n.s.
1.2 mmol l ⁻¹ Ca ²⁺	—	14.1 $\pm$ 1.0	
	+	8.6 $\pm$ 1.9	P < 0.05
3.6 mmol l ⁻¹ Ca ²⁺	—	14.5 $\pm$ 0.5	
	+	11.4 $\pm$ 0.7	P < 0.01

The tubules were preincubated for 6 h in media with different calcium concentrations. The tritiated 25 HCC was added after the preincubation period and the conversion to 1,25 DHCC assessed 15 min later. 1,25 DHCC was added in 5 μ l ethanol, and an equal volume of ethanol was added to tubes not containing 1,25 DHCC. Incubation, extraction, separation and counting of the radioactive metabolites were performed as described previously¹⁰ except that an incubation volume of 1.5 ml was used. It is important to note that the tubules remained biochemically viable for at least 6 h, as assessed by ATP levels after different periods of incubation (15 min, 1.91 μ g mg⁻¹ protein; 1 h, 2.09 μ g mg⁻¹; 3 h, 1.44 μ g mg⁻¹; 6 h, 1.68 μ g mg⁻¹) and ¹⁴CO₂ production from [U-¹⁴C] glucose which was linear for 8 h. Statistical comparisons were made by the unpaired Student's *t* test. * Not significant.

Competitive inhibition was eliminated in several ways. There is a time lag between exposure of the tubules to 1,25 DHCC and inhibition of 25 HCC-1-hydroxylase activity (Table 1a). In this and other experiments it was found that a minimum of 150 min preincubation in 1,25 DHCC was necessary before inhibition of 25 HCC-1-hydroxylase activity was apparent. This time lag was not due to delay in entry of 1,25 DHCC into the cell, as in broken cell preparations (homogenates), 1,25 DHCC in concentrations up to 125 nmol l⁻¹ (50 ng ml⁻¹) had no acute effect on 25 HCC-1-hydroxylase activity (our unpublished observations). Finally, as seen in Fig. 1, the possibility of the observed inhibition being competitive in type was completely eliminated by showing that the effect of 25 nmol l⁻¹ of 1,25 DHCC on the rate of the reaction was not reduced by increasing the substrate concentration from 12.5 to 150 nmol l⁻¹.

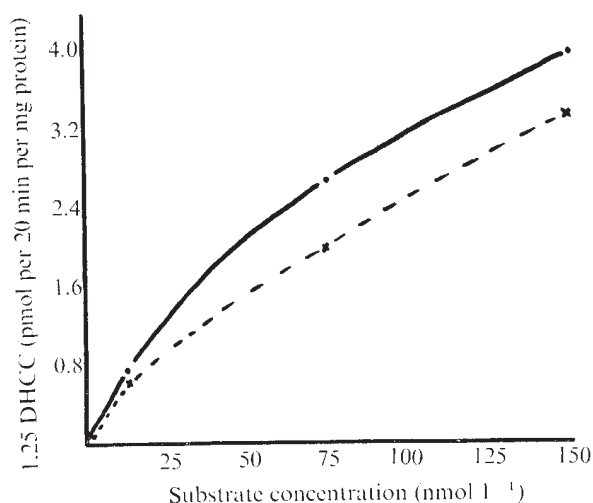


Fig. 1 Relationship between substrate concentration and the rate of the conversion of 25 HCC to 1,25 DHCC in isolated chick renal tubules. —, after 6 h preincubation in buffer and ---, after 6 h preincubation in buffer containing 25 nmol l⁻¹ of 1,25 DHCC. In the presence of 1,25 DHCC, the rate of the conversion was reduced at each substrate concentration. In this experiment, biosynthetically prepared 1,25 DHCC was used (prepared and donated by Dr E. B. Mawer, Department of Medicine, University of Manchester), and its purity was checked by high speed liquid chromatography (E. W. Matthews *et al.* unpublished).

The possibility that the time lag in onset of the inhibitory effect of 1,25 DHCC was due to a nuclear interaction with the renal cell responsible for the 25 HCC-1-hydroxylase activity was investigated by studying the effect of actinomycin D. This antibiotic is a relatively specific inhibitor of DNA-directed RNA transcription^{12,13}. The concentration used (5.4 µmol l⁻¹) inhibited 97% of 5-³H-uridine incorporation into acid-precipitable RNA after 30 min exposure of the tubule cells. Exposure to actinomycin D for 6 h did not increase 1,25 DHCC production, but it did abolish the inhibitory effect of exposure of the tubules to 1,25 DHCC for 5.5 h (Table 2). Although experience has taught caution in the interpretation of the results of experiments using inhibitors of RNA or protein synthesis, the facts that basal 25 HCC-1-hydroxylase activity was not reduced, and ¹⁴CO₂ production from [U-¹⁴C] glucose is not affected, suggest that actinomycin D was not acting as a non-specific toxin. The results support the concept that the inhibitory effect of 1,25 DHCC on its own formation might be mediated by a nuclear effect of this steroid hormone in initiating new RNA transcription.

A nuclear effect of 1,25 DHCC could not lead to inhibition of 25 HCC-1-hydroxylase activity by preventing the formation of the messenger RNA for the 25 HCC-1-hydroxylase enzyme, because virtually complete interruption of all new RNA formation by actinomycin D did not depress the enzyme activity.

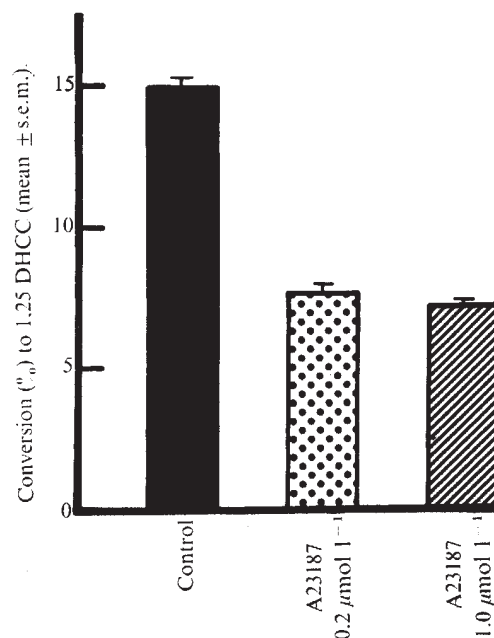


Fig. 2 Effect of the calcium ionophore A23187 on the conversion of 25 HCC to 1,25 DHCC. The preparation was preincubated for 15 min before the addition of tritiated 25 HCC and a further 15 min incubation. Cellular respiration, as assessed by ¹⁴CO₂ production from [U-¹⁴C] glucose, was unaffected by the ionophore.

The most likely explanation is that, as in the intestine^{14,15}, 1,25 DHCC increased the synthesis of proteins concerned with calcium transport¹⁶. This could result in inhibition of the 1-hydroxylase enzyme, which is known to be sensitive to calcium ion *in vitro*^{8,9}. The demonstration of a vitamin D-dependent calcium-binding protein in the kidney is consistent with this view¹⁷.

The possibility that calcium ion influx could account for inhibition of 25 HCC-1-hydroxylase activity in intact cells was tested by studying the effect of the ionophore A23187¹⁸. This agent forms lipophilic complexes with calcium (and magnesium) ions, allowing their rapid entry into cells. This agent markedly inhibited 25 HCC-1-hydroxylase activity (Fig. 2), presumably by promoting calcium ion entry. Finally, the experiment shown in Table 1 (b) demonstrated that the inhibitory action of 1,25 DHCC was abolished by lowering the extracellular calcium ion concentration of the medium.

Figure 3 summarises the scheme proposed for regulation of 25 HCC-1-hydroxylase activity at the cellular level. Reabsorption of the calcium from the tubular lumen is enhanced by 1,25 DHCC, presumably by its effect on the synthesis of proteins involved in calcium transport. However, if the calcium concentration in the tubular lumen at the site critical for the hydroxylation of 25 HCC is low, 1,25 DHCC production is unaffected. But if the renal tubular calcium rises, sufficient calcium may be reabsorbed to suppress 1,25 DHCC production. This effect on

Table 2 Prevention by actinomycin D of the effect of 1,25 DHCC on conversion of 25 HCC to 1,25 DHCC in chick kidney tubules

Agent	Recovered radioactivity (%) as 1,25 DHCC (Mean ± s.e.m.)	Significance
Control	18.5 ± 1.3	
1,25 DHCC (25 nmol l ⁻¹)	12.0 ± 1.4	P < 0.01
Actinomycin D (5.4 µmol l ⁻¹)	17.9 ± 0.6	n.s.*
Actinomycin D (5.4 µmol l ⁻¹) + 1,25 DHCC (25 nmol l ⁻¹)	17.1 ± 1.0	n.s.

Actinomycin D was added 30 min before 1,25 DHCC; there was a further 5.5 h preincubation prior to the addition of tritiated 25 HCC. In addition actinomycin D (5.4 µmol l⁻¹) does not impair ¹⁴CO₂ production from [U-¹⁴C] glucose by the tubules at 3 h or 6 h incubation (unpublished results).

* Not significant.

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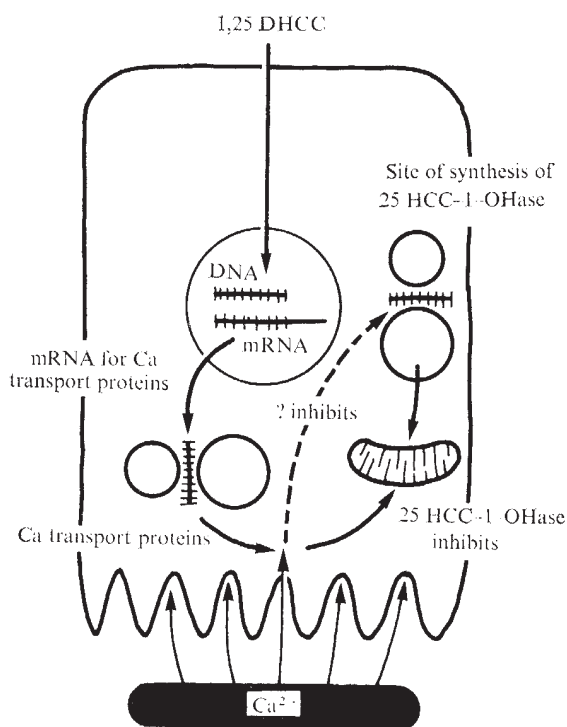


Fig. 3 Schematic representation of a renal tubule cell showing proposed action of 1,25 DHCC on reabsorption of calcium and 25 HCC-1-hydroxylase activity. Other actions of 1,25 DHCC on the tubule cell, such as induction of another hydroxylase enzyme, 25 HCC-24-hydroxylase, are also possible, but are not shown as our *in vitro* experiments gave no direct evidence for them. Similarly an effect at the translational level may exist and is indicated by ———→

enzyme activity could be followed by a delayed action on enzyme synthesis. In the absence of 1,25 DHCC, it would be predicted that only very high plasma and urinary calcium concentrations could inhibit 25 HCC-1-hydroxylase activity. This is consistent with *in vivo* observations⁵.

Thus it is proposed that 1,25 DHCC has a dual role in the kidney. It enhances calcium conservation by increased synthesis of calcium transport proteins and, perhaps more importantly, it allows the renal cell responsible for the production of 1,25 DHCC to respond appropriately to normal or high urinary calcium concentrations by reducing the rate of formation of 1,25 DHCC.

These experiments, and the hypothesis they have led to, do not imply that other control mechanisms do not exist. It has become apparent that the regulation of 1,25 DHCC production has multifactorial control, as have most other endocrine systems, and hormonal factors may also be important. The results do however, suggest a teleological explanation for the siting of the 25 HCC-1-hydroxylase enzyme system in the kidney tubule, where it is able to sense and respond to fluctuations in calcium concentration in the main outflow site from the extracellular compartment, and to circulating 1,25 DHCC concentrations.

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Erroneous base-pairing induced by a chemical carcinogen during DNA synthesis

THE stability of binding of chemical carcinogens to DNA^{1–5} *in vivo* suggests a molecular mechanism for tumour initiation. This interaction may be mutagenic in that it may affect the accuracy with which the modified DNA is copied by cellular DNA polymerases. We now report that the modification of a synthetic polynucleotide template with β -propiolactone increases

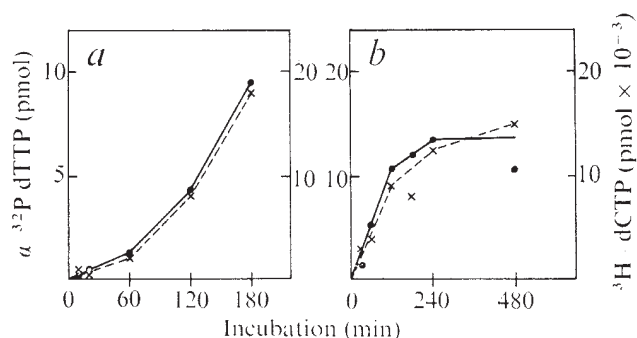


Fig. 1 Time dependency of complementary and non-complementary nucleotide incorporation. *a*, AMV DNA polymerase, 1 μ g of 85 μ M modified poly(dA)-oligo(dT) was incubated at 37°C in a mixture containing equal quantities of complementary and non-complementary nucleotide concentrations. *b*, Sea urchin nuclear DNA polymerase, 1 μ g of 43 μ M modified template was used.

The assays for simultaneous incorporation of complementary and non-complementary nucleotides are detailed in the legends to Tables 1 and 3. ●, Complementary nucleotide incorporation; ×, non-complementary nucleotide incorporation.

Table 1 Response of AMV DNA polymerase to carcinogen-modified poly(dA)-oligo(dT)

Modification of poly(dA)-oligo(dT) template	Complementary nucleotide (dTTP) incorporation (pmol)	Non-complementary nucleotide (dCTP) incorporation (pmol)	Error rate
No BPL	6.95	0.012	1/579
9 μ M BPL	5.12	0.012	1/426
43 μ M BPL	4.96	0.023	1/215
85 μ M BPL	5.50	0.027	1/203

The incorporation of complementary (dTTP) and non-complementary (dCTP) nucleotides by AMV DNA polymerase was measured at 37° C for 60 min. The reaction mixtures (total volume 0.05 ml) contained 50 mM Tris-HCl (pH 8.0), 5 mM MgCl₂, 20 mM KCl, 5 mM dithiothreitol, 2 μ g bovine serum albumin, 26 μ M α -³²P-dTTP (20 d.p.m. pmol⁻¹), 30 μ M ³H-dCTP (55,000 d.p.m. pmol⁻¹), 0.1 mg of AMV DNA polymerase, and 1 μ g of modified or unmodified poly(dA)-oligo(dT). Samples were washed as previously described¹⁴. The values given represent the average of triplicate determinations. The error rates were determined by simultaneous incorporation of dTTP and dCTP and varied by less than 10%.

the incorporation of non-complementary base-paired nucleotides into DNA with the DNA polymerase from either avian myeloblastosis virus (AMV) or sea urchin embryos. Homogeneous AMV DNA polymerase and partially purified sea

final concentration of 43 μ M one molecule was bound per 180 nucleotide residues in the template. All radioactivity after dialysis was acid-precipitable. As a control, poly(dA)₂₀₀₀-oligo(dT)₁₂₋₁₈ was incubated without β -propiolactone; and the modified and unmodified poly(dA)₂₀₀₀-oligo(dT)₁₂₋₁₈ were dialysed together. Size distributions of the unmodified and carcinogen-modified templates were similar as determined by zonal sedimentation in neutral sucrose gradients.

The accuracy of DNA synthesis with AMV DNA polymerase and modified poly(dA)₂₀₀₀-oligo(dT)₁₂₋₁₈ was determined (Table 1). Synthesis was measured by the simultaneous incorporation of the complementary (α -³²P-dTTP) and non-complementary (³H-dCTP) nucleotides. As previously reported, AMV DNA polymerase incorporates an unusually large number of non-complementary nucleotides¹⁴. With poly(dA)₂₀₀₀-oligo(dT)₁₂₋₁₈ as a template, AMV DNA polymerase incorporates one molecule of non-complementary nucleotide (dCTP) for approximately every 600 molecules of complementary nucleotide polymerised. We found that the number of non-complementary nucleotides incorporated increased progressively as template modification was increased. This error rate of 1/579 increased to 1/203 as a result of treatment with β -propiolactone. Modification with increasing amounts of β -propiolactone did not significantly decrease the incorporation of complementary nucleotides. Although the absolute error rates varied as much as twofold in experiments with different preparations of enzyme, a relative increase in error rates with carcinogen treatment similar to that shown in Table 1 was observed in all experiments.

The reaction requirements for the incorporation of non-complementary nucleotides with carcinogen-modified templates are shown in Table 2. The incorporation of non-complementary nucleotides was totally dependent on Mg²⁺, correct nucleotide (dTTP), enzyme and template. Elimination of any of these components eliminated detectable incorporation of non-complementary nucleotide. These requirements are similar to those reported for unmodified templates¹⁴ and suggest that polymerisation is required for the incorporation for

Table 2 Reaction requirements for non-complementary nucleotide incorporation with modified templates

Enzyme	Reaction mixtures	pmol Incorporation	
		Correct (dTTP)	Incorrect (dCTP)
AMV DNA polymerase	Complete	22.5	0.048
	Minus Mg ²⁺	< 1.0	< 0.001
	Minus dTTP	< 1.0	< 0.001
	Minus enzyme	< 1.0	< 0.001
	Minus template	< 1.0	< 0.001
Sea urchin nuclear DNA polymerase	Complete	< 20.6	< 0.0175
	Minus Mg ²⁺	< 1.0	< 0.002
	Minus dTTP	< 1.0	< 0.002
	Minus enzyme	< 1.0	< 0.002
	Minus template	< 1.0	< 0.002

The complete reaction mixtures and incubation conditions are given in Tables 1 and 3. The absolute error rates of AMV DNA polymerase and sea urchin DNA polymerase should not be compared because partially purified sea urchin enzyme was used in these studies.

urchin nuclear DNA polymerase, which are devoid of nuclease activity^{6,7} were used. β -Propiolactone is a potent mutagen^{8,9} and carcinogen, initiating tumours in mice¹⁰, rats¹¹ and hamsters¹². It is an alkylating agent that reacts with guanine and adenine nucleotides in DNA¹³.

β -Propiolactone (0–85 μ M final concentration) was incubated with 200 μ g of poly(dA)₂₀₀₀-oligo(dT)₁₂₋₁₈, mass ratio 1:1, for 20h at 37° C in the dark. Unbound β -propiolactone was removed by dialysis for 24h at room temperature. Studies with ³H- β -propiolactone (Searle, Amersham) demonstrate that at a

Table 3 Response of sea urchin nuclear DNA polymerase to carcinogen-modified poly(dA)-oligo(dT)

Modification of poly(dA)-oligo(dT) template	Complementary nucleotide (dTTP) incorporation (pmol)	Non-complementary nucleotide (dCTP) incorporation (pmol)	Error rate
No BPL	37.36	0.0097	1/3852
9 μ M BPL	56.31	0.023	1/2448
43 μ M BPL	33.68	0.016	1/2105
85 μ M BPL	17.89	0.011	1/1626

Complementary (dTTP) and non-complementary (dCTP) nucleotide incorporation by sea urchin nuclear DNA polymerase was measured at 28° C for 8 h. The reaction mixture (0.05 ml total volume) contained 40 mM Tris-HCl (pH 8.0), 4 mM MgCl₂, 6 mM dithiothreitol, 50 mM α -³²P-dTTP (38 d.p.m. pmol⁻¹), 50 mM ³H-dCTP (2.74 $\times$ 10⁵ d.p.m. pmol⁻¹), 10% glycerol, 3 μ g sea urchin nuclear DNA polymerase, and 1.5 μ g of modified or unmodified poly(dA)-oligo(dT). Samples were washed according to the procedure of Battula and Loeb¹⁴. The enzyme used in this experiment corresponds to Fraction V as previously reported¹⁵.

non-complementary nucleotides. The time dependency of non-complementary nucleotide incorporation was also investigated (Fig. 1a). There was coordinate incorporation of non-complementary and complementary nucleotides, suggesting that these errors are evenly distributed during synthesis. The product of the reaction was digested by snake venom phosphodiesterase. Chromatographic analysis demonstrated that the ^3H and ^{32}P radioactivity in the product was recovered as ^3H -cCMP and ^{32}P -dTMP. This indicates that the ^3H count in the product is indeed dCMP and that the non-complementary nucleotide is present in phosphodiester linkage.

To test whether this increase in errors in DNA synthesis was unique to AMV DNA polymerase, we also used sea urchin nuclear DNA polymerase. As Table 3 shows, the error rate with unmodified poly(dA)-oligo(dT)₁₂₋₁₈ was 1/3852 and could be increased to 1/1626 with more extensive template modification. When the template was treated with the carcinogen there was an initial stimulation in synthesis followed by a decrease in synthesis as template modification increased. The incorporation of the non-complementary nucleotide was dependent on Mg^{2+} , correct nucleotide, enzyme and template (Table 3). The time dependency of the reaction (Fig. 1b) shows that continued synthesis is required for incorporation.

These results demonstrate a possible relationship between a chemical carcinogen and errors in DNA replication. The accuracy with which a polynucleotide template is copied by AMV DNA polymerase and by sea urchin DNA polymerase is decreased as a result of modification of the template by the carcinogen. Even though the stoichiometry of bound β -propiolactone approximates the increased number of errors observed with AMV DNA polymerase, we have no direct evidence that the alkylated nucleotides are in juxtaposition to the observed errors in the newly synthesised DNA. Thus, a chemical carcinogen may potentiate the mutagenic effects of the AMV DNA polymerase and may directly induce mutations during DNA replication in normal cells.

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Early simultaneous appearance of antigen binding cells in the foetal sheep

THE two classes of theory on the generation of diversity in the immune system differ as to when the genetic capacity for producing a full repertoire of antibody clonotypes is achieved. The germline theory¹ proposes that diversification occurs mainly on an evolutionary timescale at the species level, with the immune capacity of an individual predetermined at the instant of fertilisation; the various somatic theories²⁻⁸ propose a much briefer period of diversification from a minimal complement of V genes, occurring during the ontogeny of the individual or even later in life. We have attempted to study the time course of generation of diversity by following the appearance of lymphoid cell receptors binding any of several antigens in foetal sheep.

If one assumes that cell-surface receptors for antigen reflect accurately the antibody specificities produced by the cells, then, according to the germ-line theory, one prediction would be that all receptor specificities should arise simultaneously as soon as the immune system had developed to the point where the receptors could be displayed on the surfaces of lymphoid cells. According to somatic theories, the appearance of various receptor specificities might follow several different patterns. (a) If diversification occurred randomly, then in any one animal there should be a random accumulation of receptor specificities following no set time course, except for the early expression of certain high probability 'hot-spot' mutations or genetic changes few steps removed from the original, small germ-line set of V genes. (b) If diversification were antigen driven, then the number of receptor specificities and the frequency of antigen-binding cells (ABC) for related antigens should increase with antigen exposure. (c) Another possibility would be that primitive antigen receptors, which bind weakly to a large variety of antigens, would be replaced, as genetic diversification occurred, by receptors which would discriminate much more precisely among different epitopes.

Silverstein has reported⁹ that the maturation of immune competence in the foetal sheep, as measured by the animal's ability to make antibody, does not occur at the same stage of development for all antigens, but follows a particular sequence: anti- ΦX174 antibody can be made at day 35 of gestation, anti-ferritin at day 65, anti-ovalbumin at day 125, and antibodies to *Salmonella* and BCG only several weeks after birth (where full term is about 150 d). A hierarchy of responsiveness to nine bacteriophage, hapten and protein antigens has also been described in the opossum¹⁰. The stage of development is quite different, however, at which the opossum first responds to two antigens also used in the sheep studies. The events leading up to antibody synthesis include the interaction of T and B lymphocytes and macrophages; therefore, a lack of response could be due to a deficiency of receptor-generating cells or essential accessory cells⁹.

We chose to look for cells capable of specifically binding antigen, among which population the precursors of antibody-forming cells and T helper cells would be expected to be found, to see how early in ontogeny such cells were present in the foetal sheep and whether their appearance paralleled the appearance of response capacity measured by antibody production. It has already been demonstrated that the sheep has a proportion of Ig-bearing lymphocytes in its peripheral lymphoid organs similar to that in the mouse¹¹, and we have confirmed this with our fluorescent anti-sheep Ig reagent. Since the sheep has a placental barrier to maternal immunoglobulin⁹, the appearance of foetal antigen-binding cells (ABC) should reflect the genetic capacity of the foetus to make specific antigen recognition units. Single cell suspensions were prepared from the lymphoid organs of foetuses from time-bred sheep at 58, 87, and 140 d gestation, and from the spleens of two of the ewes. The cells were fixed with glutaraldehyde to preserve their receptors¹²⁻¹⁴, incubated with 500 $\mu\text{g ml}^{-1}$ fluorochrome-conjugated antigen, and observed for ABC with a Leitz Ortholux

fluorescence microscope equipped with Ploem-type vertical illumination¹⁵. (For a more detailed presentation of the methods, see DeLuca *et al.* (submitted for publication)).

Table 1 shows the % ABC observed in the spleens of the foetal and adult (pregnant) sheep. ABC were observed for all four antigens as early as day 58 of gestation, including ovalbumin for which no antibody formation can be detected until 125 d gestation. Splenic ABC were always Ig-bearing cells; this was only examined in a 140-d foetal sheep and its mother. No dramatic increase in ABC is seen as the foetus develops. ABC frequencies are higher in the adult spleen (especially in the 140-d pregnant ewe), perhaps as a result of exposure to cross-reacting antigens, a change in the cell types constituting the spleen, or changes associated with pregnancy as observed in the mouse¹⁶. Binding of HSF, OA, and GZ were shown to be non-competitive by inhibition experiments in which a 10-fold excess of HSF or a 40-fold excess of OA prevented the binding of rhodamine-HSF or fluorescein-OA, respectively, but not the binding of fluorescein-GZ. Fluorescein-HSF and rhodamine-HSF gave the same frequency of ABC with adult spleen cells.

ABC for GZ and HSF were observed in the thymus, marrow and liver as well as the spleen of the 58-d foetal sheep (Table 2). Two differences from ABC frequencies in foetal mice¹⁶ are readily apparent. First, the ABC frequency in the foetal sheep liver is very low, and many ABC are present in the marrow; even late in gestation, foetal mice have 0.1–0.5% ABC in the liver and virtually no cells in the marrow. Foetal sheep liver contains many large hepatocytes at 58 d, contrasted to foetal

of B or T lymphocytes which can recognise antigen. No evidence exists for a sequential appearance of ABC for different antigens; although foetal sheep first respond to HSF at day 65 of gestation and to OA at day 125, ABC for both antigens are present on day 58. In these early ABC, the inhibition of specific antigen binding by nonlabelled homologous antigen and its absence of effect on the binding of unrelated antigens is not compatible with the concept of a primitive receptor, capable of binding many different epitopes.

Table 2 ABC in several lymphoid organs of 58 d foetal sheep

Organ	%ABC*	
	GZ	HSF
Thymus	0.05	0.09
Marrow	0.60	1.0
Spleen	0.08	0.14
Liver	<0.02	0.009

* At least 10 ABC or 10,000 total cells were counted for most samples.

Table 1 Splenic antigen-binding cells in foetal and adult sheep

Age	GZ†	% ABC*			OA§
		HSF‡	KLH‡		
58 d	0.08	0.14	0.13		0.04
87 d	0.08	0.06	0.03		0.07
140 d	0.10	0.10	0.11		0.20
Adult ¶	0.54	1.0	0.35		0.25
Adult ¶¶	2.9	2.4	2.8		0.8

* At least 10 ABC or 10,000 total cells were counted for most samples.

† *E. coli* β -galactosidase, extracted from strain A-3245 and purified by affinity chromatography.

‡ Horse spleen ferritin and keyhole limpet haemocyanin, A grade (Sigma).

§ Ovalbumin, 5 $\times$ crystallised (Pentex).

¶ 58 d pregnant.

¶¶ 140 d pregnant.

Cell preparations, antigen incubation, and washes were done in buffered balanced salt solution, pH 7.2, containing 0.25% gelatin. Cells were fixed with 0.2% glutaraldehyde in 0.1 M phosphate buffer pH 7.0 for 15 min; antigen incubation was for 30–60 min. All procedures were carried out in the cold. The sheep were obtained from Western Scientific Breeders, Inc., Davis, California.

The early appearance of ABC for a variety of antigens in mice and man^{16,18,19}, as well as the sheep, argues against a requirement for a long period of somatic diversification to produce most of the receptor clonotypes the individual possesses. It is possible that the use of three large antigens might exaggerate the proportion of the receptor universe present at the earliest time. But our studies reported here with ovalbumin, and experiments with early foetal binding of horse radish peroxidase¹⁸ and flagellin¹⁸ seem to vitiate this objection.

We conclude that the ability to bind antigen is one of the earliest events in immune development, and that the genetic potential to respond to a wide variety of epitopes is predominantly germline in nature. The apparently orderly development of the capacity to respond to different antigens by antibody production must reflect a biological clock which modulates T cell–B cell–macrophage interactions and not the regulated acquisition of particular *V*-gene regions.

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mouse liver which contains mostly haematopoietic stem cells. The second developmental difference is in the thymus. Mice have high ABC frequencies (of the order of 1%) when the thymus can first be studied (14 d = 70% term), declining to 0.1% by late gestation and birth. At 58 d (40% term), 20 d after thymocytes first are observed in the thymic rudiment¹⁷, the thymic ABC frequency in the foetal sheep is quite low. It may be that a decline in ABC frequency similar to that observed in mouse and human foetal thymus^{16,18,19} has already occurred by 58 d gestation in the sheep.

The data presented above suggest that the inability of the foetal sheep to make an antibody response to all antigens early in development is probably a result of a deficiency in antigen handling or cell interactions, a requirement which may be more stringent for some responses than others, rather than to a lack

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Presence of J chain in human immunocytes containing various immunoglobulin classes

THE polypeptide J chain is common to dimeric IgA and polymeric IgM^{1–3}. Immunocytes producing such immunoglobulins have been shown to synthesise J chain^{4–6}; its incorporation has therefore been assumed to be an essential step in the intracellular joining of the monomer (H₂L₂) subunits. But the finding of a monoclonal polymeric IgM lacking J chain^{7,8}, and reassociation of IgM subunits depleted of J chain⁹, have led to questioning of its role as an initiator of polymerisation. Experiments demonstrating J chain in four murine IgG myeloma cell lines¹⁰ prompted me to examine its cellular localisation in human biopsy material. Cytoplasmic J chain was detected in IgD, IgM, IgG, and IgA immunocytes by direct immunofluorescence. This localisation was compared with the cytoplasmic ability to bind "secretory component" (SC) *in vitro* in a SC-affinity test that has been postulated to distinguish between polymer and monomer producing cells¹¹.

Human J chain was purified from dimeric IgA by reduction with 10–20 mM dithiothreitol, gel filtration and immuno-adsorption with an insolubilised antiserum to human serum¹². Four rabbits each received subcutaneous injections of 0.4 mg J chain coupled to bovine serum albumin (BSA)¹³ and emulsified in Freund's complete adjuvant. The same dose was given after 4 weeks; and 1 month thereafter 1 mg uncoupled J chain was injected. All rabbits had an appreciable antibody titre 14 d subsequently. After adsorption with BSA and insolubilised serum from a patient with hypo- γ -globulinaemia, the antisera were shown to be monospecific to J chain, giving an identity reaction with reference antiserum provided by Dr J. Mestecky. IgG from the most potent antiserum was labelled with fluorescein isothiocyanate, and a fraction of this 'green' conjugate with an absorbance ratio of 2.2 was used at 0.6 mg and 1/10 precipitating units per ml (ref. 14). Human tissue specimens were extracted for 48 h at 4°C in isotonic phosphate-buffered saline, fixed in 95% alcohol, and embedded in paraffin¹⁵. Serial sections were incubated with a combination of the 'green' anti-J-chain and a 'red' (rhodamine) conjugate monospecific to human IgG, IgA, IgM or IgD^{14,15}. Other sections were first incubated with SC, and thereafter with a combination of 'red' anti-SC and 'green' anti-Ig to identify cells with cytoplasmic affinity for SC^{11,16}. The sections were examined in a Leitz Ortholux microscope equipped with a Ploem-type vertical illuminator and interference filters for narrow-band excitation and selective filtration of green or red fluorescence¹⁵.

The SC affinity test distinguished between three types of tissue with regard to IgA cells: (1) immunocytes associated with glands in colon and nasal mucosa were generally positive¹¹;

(2) tonsils contained chiefly negative IgA immunocytes but also positive cells scattered or in groups¹¹; and (3) IgA cells in inflammatory infiltrates did not generally bind SC. The most 'pure' cell population of the latter type was found in the gingiva which is a part of the oral mucosa completely lacking glands. This tissue is prone to chronic inflammation because of contact with the bacterial plaque on the teeth. IgM immunocytes associated with glands, also bound SC, whereas the few such cells present in the gingiva were equally positive or negative. We interpreted these findings as indicating that IgA and IgM immunocytes of glandular areas produce dimeric or polymeric immunoglobulins containing J chain. This assumption was based on the fact that isolated dimeric or polymeric IgA and 19S IgM combine spontaneously with SC in test tube experiments; but apparently only when the immunoglobulins contain J chain^{8,16}. Monomers, including 7S IgA, do not combine with SC. By inference most IgA cells in the gingiva should produce monomeric IgA.

These conclusions were supported by the direct immunohistochemical demonstration of J chain. In the gingiva only a few (3–8) out of many IgA cells in each section contained this peptide (Fig. 1a), a number comparable with that of SC-binding cells. J chain could moreover be detected in only about 50% of the few gingival IgM immunocytes. This contrasts with glandular areas in the nasal mucosa and colon where most IgM (Fig. 1d) and IgA cells were positive. The intensity of the IgA cells was generally weak and varied considerably, however, possibly reflecting different concentrations of this peptide (Fig. 1e). In the tonsils small groups of cells containing J chain especially beneath the epithelium, corresponded to areas where some SC-binding IgA immunocytes were present. These results substantiated that IgA cells appearing in inflamed gingiva and tonsils are chiefly monomer producers which cannot synthesise J chain.

Many IgG cells characteristically appear in foci of chronic inflammation such as in the gingiva^{17,18} and a few (<1%) definitely contained J chain (Fig. 1b). In the superficial stroma of nasal mucosa IgG immunocytes accumulate as a result of rhinitis¹⁹; 30–60% of these cells were J chain producers (Fig. 1c). Also many of the few IgG immunocytes adjacent to the crypts in normal colon mucosa were J chain-positive. This was also true for IgG cells found along with IgM cells in some of the lymphoid follicles in the tonsils. But, for two out of the four specimens (one pharyngeal and one palatine) the number of positive cells was too large to be accounted for by IgM, IgG and IgA immunocytes (Fig. 1f). In these two specimens cells containing IgD outnumbered those with IgM, especially between the lymphoid follicles and the surface; and the majority of the IgD immunocytes were indeed J chain-positive.

The specificity of the J chain immunofluorescence was established as follows: (1) Addition to the conjugate of highly purified J chain (1 mg ml⁻¹) blocked the staining reaction completely; (2) addition of comparable amounts of F(ab')₂ had no effect; (3) the simultaneous demonstration of J chain positive and negative immunocytes of each Ig class excluded the possibility of cross reactions with parts of the L or H chains. Furthermore, none of our conjugates produced fluorescing cells when applied in working dilutions to sections of a nasal polyp from a patient with hypo- γ -globulinaemia.

We have thus for the first time provided evidence that J chain may be produced by immunocytes of any Ig class with the possible exception of IgE. This accords with several studies indicating that B cells of a specific clone are genetically multipotent with regard to their H chain product²⁰. Many circulating B cells have both IgM and IgD in their surface membranes^{21,22}; and the simultaneous presence of IgM in the membrane and IgG in the cytoplasm has also been described²³. In general, however, the secretory product of a single mature B cell is of only one Ig class and it has been postulated that there may be an intraclonal switch from expression of IgM synthesis to IgG, and perhaps to IgA²⁴. The finding of IgD-containing plasma

cells adjacent to lymphoid follicles indicates that IgD may be expressed early in such a development. The presence of J chain in most of these cells moreover suggests that this polypeptide represents a very basic gene product of B cells, the synthesis of which is repressed only after prolonged clonal proliferation. In lymphoid follicles and adjacent to glandular epithelia, continuous clonal expansions from immunocytes that have recently switched their H chain product may occur as a result of frequent presentation of new antigenic material. This is in

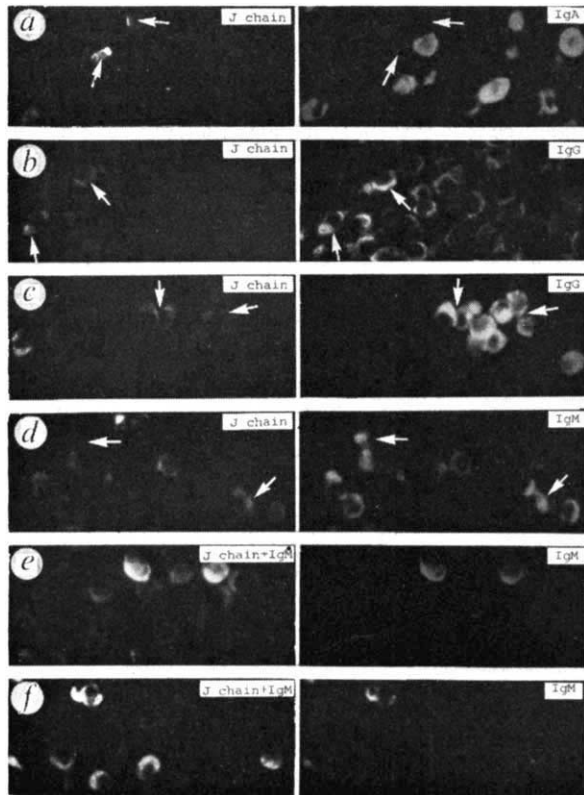


Fig. 1 Immunohistochemical demonstration of J chain in human tissues. Paired staining was performed with a 'green' anti-J-chain conjugate combined with a 'red' conjugate specific for IgA, IgG or IgM. Right column: records of selective red emission. Left column: a-d, records of selective green emission; e, f, double exposures. a, Field from gingival section with two intense J chain-positive cells (arrows) which are not IgA immunocytes. b, Comparable field from section adjacent to a showing a few J chain-positive IgG immunocytes (arrows). c, Area beneath the surface epithelium of nasal mucosa containing two intense (left arrow) and three faint (right arrow) J chain-positive IgG immunocytes. d, Field from glandular area of inflamed colon mucosa (Crohn's disease) containing numerous J chain-positive IgM immunocytes. Left arrow, indicates a negative cell, and right arrow, a positive one. e, Field from glandular area of normal colon mucosa with two IgM cells which are stained brightly for J chain (mixed colour in left picture), and numerous more or less faint, green cells which in neighbouring section were shown to be IgA immunocytes. f, Part of an area adjacent to a tonsillar lymphoid follicle. There were a few J chain-positive IgM cells (two shown), and several other immunocytes brightly fluorescing for J chain. Staining of neighbouring sections demonstrated that most of these contained IgD, and some contained IgG.

accordance with the fact that the largest number of J chain-positive IgG cells were found in these areas.

Studies of mouse and human cell lines have provided contradictory information with regard to the intracellular events in the Ig polymerisation process^{5,25,26}. The SC-affinity test directly

demonstrates that normal gland-associated human IgA and IgM immunocytes must contain cytoplasmic subunits in an arrangement with J chain comparable to that constituting the SC-binding site of the extracellular immunoglobulins¹⁶. For IgM³, as well as IgA, this arrangement may be a dimer which is not necessarily covalently stabilised initially. If this dimeric configuration is likewise expressed in the membrane of IgM and IgA cell precursors, these B lymphocytes may by contact with SC-producing epithelial cells or interstitial free SC, receive a stimulus on their mucosal recirculation route, which induces their settlement and local proliferation. In addition the J chain-dependent SC-binding site of extracellular immunoglobulins may mediate the epithelial reception and the selective glandular transmission of dimeric IgA and 19S IgM^{15,27}. This peptide therefore seems to be important in the evolution of the mucosal immune system.

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Anti-tumour cytotoxic effects mediated by minor and major cell populations of lymph nodes

SEVERAL studies have demonstrated that cell-mediated immunity to animal and human tumours can be measured *in vitro* by colony inhibition or microcytotoxicity tests in which immune lymphoid cells kill and/or inhibit the growth of tumour cells^{1,2}, and more recent studies have attempted to extend these results by measuring the relative contributions of T and B lymphocytes to the anti-tumour effects noted^{3,4}. The principle upon which these latter experiments have been based is that selective depletion of one or the other lymphocyte subpopulation, followed by measurement of the capacity of the residual population to produce an effect, should indicate which subpopulation is operative in the system. But, conclusions drawn from such experiments are critically dependent upon the assumption that the remaining lymphocyte subpopulation is not contaminated with other cell types; or, if it is, the contaminating cells are unlikely to be effector cells in the assay used because they are inactive and/or infrequent. Acceptance of this assumption requires careful evaluation; for as we have observed in the present investigation of effector cells operating during the primary immune response to murine Moloney sarcomas, a cell type of low frequency may contribute in a major way to the cytotoxic effects noted *in vitro*. Furthermore, these cells are present in considerable numbers within regressing tumours *in vivo*.

Table 1 Cytostasis mediated by LNC and non-adherent LNC

Effector cells	MSC		MCA-12	
	c.p.m.	%*	c.p.m.	%*
LNC				
Normal	7,532±865	62†	9,559±700	29†
Immune	2,814±378		6,733±530	
Non-adherent LNC				
Normal	14,043±1288	44†	12,869±1852	7(ns)
Immune	7,748±605		11,940±1189	

MSC or MCA-12 tumour cells (800) were cultured for 42 h with 8×10^5 effector cells in Linbro Multidish Disposo trays (0.2 ml per well); trays were washed by immersion, surviving tumour cells were pulsed for 6 h with IUdR (0.2 μ Ci per well), and trays were washed again. For effects of varying tumour cell and effector cell number in the assay see ref. 6.

LNC were used as such or were passed through divinylbenzene/polystyrene bead columns (37°C) to remove adherent LNC⁶. Non-adherent LNC preparations contain <0.2% phagocytic macrophages and >99% lymphocytes of which 20–25% are Ig and 65–75% are θ -bearing cells.

CPM IUdR (mean $\pm$ s.d.) remaining adherent at the conclusion of the cytotoxicity test.

*Percent cytostasis (or cytotoxicity) =
 (c.p.m. in cultures with normal effector cells) – (c.p.m. in cultures with immune effector cells)

(c.p.m. in cultures with normal effector cells) $\times 100$

Percentages evaluated by Student's *t* test: ns = not significant; †*P* < 0.001.

Moloney sarcomas were induced in 4-week-old female BALB/c mice; and 12 to 18 d later, when tumours were regressing, lymph node cell (LNC) suspensions were prepared and their effects on syngeneic tumour cells were determined as previously described⁵⁻⁷. Cell-mediated killing of tumour

cells (cytotoxicity test) was measured by labelling *in vitro* lines of tumour cells with ¹²⁵I-iododeoxyuridine (IUdR), a DNA isotope, and culturing them with effector cells for 48 h. Cell-mediated inhibition of tumour cell growth (cytostasis test) was assessed similarly except that unlabelled tumour cells were cultured with effector cells for 42 h and then surviving tumour cells were pulsed with IUdR for 6 h. In both assays, the number of surviving adherent tumour cells correlates with the amount of radioactivity remaining adherent to the culture surface⁶. We have recently made an extensive analysis of the variables influencing the results obtained in this assay system, and the work reported here is based on these findings⁶.

Whole LNC obtained at the time of tumour regression killed both Moloney sarcoma cells (MSC) and unrelated tumour cells such as polyoma virus-transformed 3T3 fibroblasts (PY)⁷ and methylcholanthrene-induced sarcoma cells (MCA-12)⁸. A representative cytostasis test in which MSC or MCA-12 were cultured with LNC demonstrates that both kinds of tumour cells were killed by LNC from mice whose tumours were regressing (Table 1). PY and MCA-12 tumour cells are unrelated antigenically to MSC by the following criteria: (1) serum from mice with regressing Moloney sarcomas binds to MSC but not to PY and MCA-12 as detected by ¹²⁵I-rabbit anti-mouse immunoglobulin (Seeger, unpublished); and (2) neither PY nor MCA-12 has C-type particles or group-specific

Table 2 Treatment of LNC with silica and of non-adherent LNC with anti- θ serum

Experiment	Effector cells	Treatment	c.p.m.*		%
			Normal	Immune	
1	LNC	None	3,933±254	2,568±125	34†
		Silica	3,672±682	3,144±530	14(ns)
2	Non-adherent LNC	Normal	6,808±923	4,446±427	34†
		Anti- θ +C'	5,028±396	4,426±133	11(ns)
3	Non-adherent LNC	Normal	1,059±85	679±91	35†
		Anti- θ +C'	769±96	781±85	+1 (ns)

Experiment (1): cytostasis test as in Table 1 except that 4×10^5 LNC were cultured without or with silica (40 μ g ml⁻¹ Min-U-Sil 5 μ m diameter (Whitaker-Clark-Daniels)) for 24 h before unlabelled MSC were added. Cultures were then continued for 42 h when surviving MSC were pulsed with IUdR.

Experiment (2): cytostasis test performed as in Table 1. NALNC (5×10^6) were treated with anti- θ C3H serum (raised in AKR mice by repeated injections of C3H thymocytes and used at a dilution of 1/10 which was on a plateau of killing against BALB/c LNC; specificity of antiserum for the θ alloantigen demonstrated by showing that absorption with BALB/c brain removed all cytotoxic activity) or normal AKR serum (30 min, room temperature). Both groups then were treated with guinea pig complement (1/10 dilution, 30 min, 37°C). Dead cells were removed by incubation in 0.25% trypsin in Tris buffer (5 min, 37°C) followed by filtration through a short filter (boiled cotton wool).

Experiment (3): cytotoxicity test: 2,000 IUdR-labelled MSC were cultured with 8×10^5 effector cells for 48 h. Effector cells prepared as in Experiment 2.

Percentages evaluated by Student's *t* test; ns = not significant; †*P* < 0.01; ‡*P* < 0.001.

*MSC was the target cell in these experiments.

antigen which MSC does have. Therefore, the observed cytotoxicity is likely to be truly nonspecific and not due to cross-reacting antigens.

By contrast, non-adherent LNC (NALNC) obtained from mice whose tumours were regressing killed MSC but not antigenically unrelated MCA-12 (Table 1). Note that the magnitude of killing mediated by NALNC in this and previous experiments⁶ is less than that mediated by an equal number of whole LNC. Specific killing by NALNC agrees with our previously reported observations^{6,7} and with serologically defined specificities.

We suspected that the dominant effector cells in whole LNC suspensions were macrophages, even though they account for only 2.5% of the effector cells, because: (1) activated macrophages kill nonspecifically^{8,9}; (2) nonspecific killing mediated by LNC is not abrogated when T lymphocytes are removed by treatment with anti- θ C3H serum and complement⁷; (3) macrophages are adherent cells, and removal of adherent cells abrogates nonspecific killing^{8,7}; and (4) macrophages are present in greater numbers in immune than in normal control LNC suspensions (2.5% compared with 0.9%)⁷ (proportions of macrophages were estimated from cytocentrifuge preparations following incubation with polystyrene particles as markers of phagocytosis). To further test the possibility that macrophages, although infrequent in number, were the dominant effector cells, whole LNC were cultured with silica for 24 h to kill macrophages^{7,10}; and then unlabelled tumour cells were added to the remaining LNC (Table 2, experiment 1). Because low numbers of NALNC (2×10^5 – 4×10^5) are not detectably cytotoxic in this assay system⁶, we expected that removal of macrophages from 4×10^5 LNC by treatment with silica would abrogate the cytotoxic effect. Indeed, immune LNC treated in such a manner did not significantly inhibit the growth of MSC, whereas untreated immune LNC did so. Control experiments indicated that silica was not affecting lymphocyte survival.

The specific killing observed with NALNC is most likely due to lymphocytes, for such preparations contain >99% lymphocytes and less than 0.2% macrophages. To determine if this killing was dependent on T lymphocytes, NALNC were treated with anti- θ C3H serum and complement (Table 2). In two such experiments, one a cytostasis test (experiment 2) and the other a cytotoxicity test (experiment 3), treatment with normal AKR serum and complement (control treatment) did not affect the differences between normal and immune NALNC, but treatment with anti- θ C3H serum and complement markedly decreased or completely abrogated the differences between them. We interpret this as indicating the dependence of the specific effect of NALNC on T lymphocytes. As yet we have no evidence on whether the cytotoxicity mediated by NALNC is due to direct T cell killing or is mediated by other cells whose action depends on T lymphocytes.

A number of other recent studies of the immune response to primary Moloney sarcomas have been aimed at determining which cell types mediate cytotoxic reactions *in vitro*. Lamont

*et al.*³, using a microplate system involving visual counting of surviving adherent tumour cells, have reported that immunologically specific inhibition of tumour cell growth was mediated by mixtures of whole LNC and spleen cells during the development and after the regression of Moloney sarcomas. Cell separation experiments suggested that both T and B lymphocytes were responsible for the effects noted. Plata *et al.*⁴ have reported similar results in a microplate assay, but in a ⁵¹Cr release assay the same group of workers¹¹ and others¹² have found that T lymphocytes are the only active population at the time of tumour regression. The reasons why these studies have not detected macrophage mediated effects when using whole LNC or spleen cells is unclear, for when Moloney sarcomas are regressing, LNC and spleen cell suspensions contain 2.5 and 10.1% macrophage respectively⁷. Even if preparative methods are used to remove adherent and phagocytic macrophages from spleen suspensions, we have found that non-adherent and non-phagocytic precursors soon become mature macrophages capable of cytotoxicity. Possibly the tumour cells used in previous studies^{3,4} were not susceptible to macrophage-mediated cytotoxic effects. Certainly, the relatively short term ⁵¹Cr release assay may be less able to detect macrophage effects than the 48 h test which we use.

Our experiments demonstrate that macrophages, a minor non-T cell subpopulation of lymph nodes, can be potent cytotoxic cells *in vitro* overshadowing the effects of lymphocytes. This observation suggests caution in interpreting experiments in which effects of different cell types are being assessed if such preparations are contaminated by even small numbers of other cell types. In the host response to primary Moloney sarcomas, cytotoxicity mediated by macrophages is nonspecific, whereas that mediated by lymphocytes is specific and is dependent on T lymphocytes. None the less, the cytotoxic effects of macrophages *in vivo* may be of considerable importance. Certainly, regressing Moloney tumours contain large numbers of such cells (Fig. 1). We are attempting to clarify the relative importance of macrophages and lymphocytes as effector cells in tumour rejection by studying the cytotoxic potential of cells taken directly from tumours.

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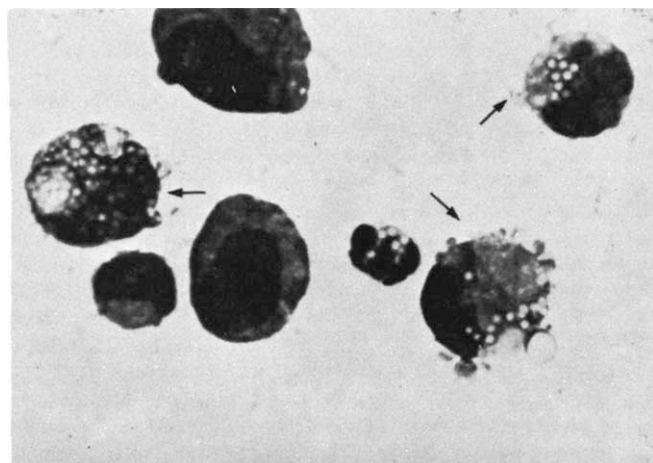


Fig. 1 Cytocentrifuge preparation made from a Moloney sarcoma. The tumour was trypsinised to provide a cell suspension which was then incubated with polystyrene beads. The beads then provide markers of phagocytic cells such as macrophages and polymorphonuclear leukocytes. Three macrophages are arrowed; the remaining cells comprise two large cells which are tumour cells and one lymphocyte and one polymorph (containing beads). Macrophages make up 35% of the cells present.

matters arising

Intestinal transport protein

We would like to take issue with the recent letter of Faust and Shearin¹ reporting on a "sugar and amino acid transport protein . . . from hamster jejunum".

The questionable significance of the observed D-glucose binding proteins from the intestine to intestinal Na⁺-dependent monosaccharide transport has been discussed in detail by Eichholz and Howell² and Garcia-Castineiras *et al.*³. In addition, we would like to point out that it is highly misleading to give a binding protein the adjunct transport when it does not fulfil one of the fundamental requirements for transport, namely high rates of binding and debinding. That the debinding of D-glucose is extremely slow has been previously noted by Eichholz *et al.*⁴, and can be deduced from the gel chromatography data of Faust and Shearin¹. In fact, D-¹⁴C-glucose remains tightly bound to a macromolecule during passage through Sephadex G-75¹. If anything, this tight binding suggests that the "protein" plays a role in storage rather than transport of D-glucose.

That the bulk of the observed D-glucose binding may be unrelated to transport is also indicated by one of our observations. We have investigated D-glucose transport in isolated and highly purified vesicles of brush border membrane from rat small intestine^{5,6}. Pre-

viously, D-glucose uptake by the isolated membranes had been measured mainly at pH 7.5. When investigating D-glucose uptake as a function of pH we noticed that with lower pH values the equilibrium uptake increased above the pH 7.5 level. This 'extra' D-glucose uptake exhibited a pronounced maximum around pH 6.0. The time curve of uptake did not seem to be altered by pH, that is, constant levels of D-glucose were always reached by 5 min incubation at 25° C. The absolute amounts of 'extra' uptake varied between 0.3 and 1.0 nmol per mg membrane protein (measured at 1 mM D-glucose) in different preparations. Bacterial contamination is not responsible for our observation.

To determine whether the 'extra' D-glucose uptake represented binding to the brush border membrane or transport into the intravesicular membrane space we measured the dependence of D- and L-glucose uptake on the osmolarity of the suspension medium at pH 7.5 and pH 6.0 (Fig. 1). D- and L-glucose uptake at pH 7.5 and also L-glucose uptake at pH 6.0 can be accounted for totally by transport into an osmotically active space (line goes through the origin). The 'extra' D-glucose uptake at pH 6.0, however, is independent of the medium osmolarity suggesting binding (300 pmol per mg protein in Fig. 1; intersection of the line with the ordinate).

The rate of D-glucose transport was found to be equally fast at all pH values between pH 8.0 and 5.0. That transport can proceed without measurable binding, at least on the scale indicated in Fig. 1, suggests to us that transport and measured binding are unrelated. At the moment, we have no hint as to function or origin of the D-glucose binding.

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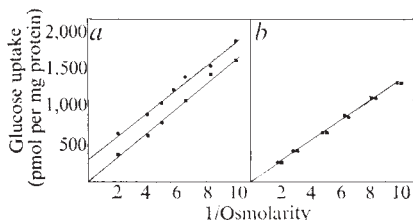


Fig. 1 Effect of medium osmolarity on D-glucose uptake in isolated intestinal brush border membranes. Preparation of membranes (in 100 mM cellobiose, 1 mM HEPES, adjusted to pH 7.5 with Tris, and 0.1 mM MgSO₄) and the uptake procedure have been previously described⁵. Uptake was measured from a medium with the following final composition: NaSCN (25 mM), Tris-HEPES (1 mM), MgSO₄ (0.1 mM), D-1-³H-glucose (1 mM) and L-1-¹⁴C-glucose (1 mM) and enough cellobiose to give the indicated osmolarity (shown as inverse osmolarity). Time of incubation: D-glucose, 10 min; L-glucose, 20 min; temperature, 25° C. The lines were calculated by regression analysis (correlation coefficient in all cases: 0.99). a, D-glucose; b, L-glucose. ■, pH 7.5; ●, pH 6.0.

DR FAUST REPLIES—Since the brush border region of the intestinal mucosal cell is a digestive-absorptive organelle, the pumping of monosaccharides and amino acids across this entire organelle must be considered in the mechanism by which these substrates are accumulated against their concentration gradients within the cytoplasm.

The phenomenon that Hopfer and Sigrist-Nelson have observed concerns the movement of D-glucose across one section of the structurally complex brush border, the plasma membrane. We agree that their observed D-glucose binding is not related to transport and we feel that it could be caused by binding to disaccharidases in the plasma membrane. Our binding protein, however, is located in the core fraction of disrupted brush borders which does not contain hydrolytic enzymes. It possesses sites for the Na⁺-dependent 'trapping' of specific monosaccharides and amino acids that are derived either indirectly from the hydrolysis of dietary oligosaccharides and polypeptides or directly from dietary monomers in the intestinal lumen. We have shown that the specificity for this binding is similar to that for the active transport of these substances by the small intestine. Furthermore, these observations could not be attributed to bacterial contamination.

We visualise that our isolated binding protein is involved in the Na⁺-dependent active transport of sugars and amino acids from the core region of the brush border into the cytoplasm. This occurs after the initial penetration of these monomers through the plasma membrane.

We concede, however, that this protein can only be regarded provisionally as a transport protein until debinding is adequately studied. Debinding of the sugar and amino acid ternary complexes could be triggered by a conformational change in the binding protein caused by the low intracellular Na⁺ concentration, the replacement of Na⁺ in the ternary complex by K⁺ from the high K⁺ intracellular region, and by a combination of these phenomena with the aid of energy derived directly from cellular metabolism. None of these conditions was prevalent in our Sephadex G-75 fractionation studies, therefore D-¹⁴C-glucose and L-¹⁴C-histidine remained tightly bound to the isolated protein subunits.

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reviews

THE focus of research into the process of technological innovations seems to have shifted recently to the developing countries. From the problems of 'high' technology and science-based industries, one now hears increasingly about transferring technology to the countries of the Third World; and it is not advanced technology but intermediate, or other appropriate, technology. This development in the language of technology transfer records the simple fact that innovation is a complex social (some would say cultural) process and that the experience of the western nations with technological developments will not transplant easily to other countries trying to make the transition to industrialisation.

The experience of western nations over the last two hundred years forms the historical backdrop to the manifold problems of making and implementing decisions intended to bring about modernisation. But modernisation, the author makes clear, involves the marriage of technology and civic life. By this he means that every developing society must be viewed as a complex of already existing and functioning social and economic processes and that no decision to introduce new technology can hope to succeed without an operational understanding of this fact. As if to guide the reader in the implications of these facts, the author painstakingly documents the industrial history of the western world

Industrial revolutions

M. Gibbons

Technology and Civic Life: Making and Implementing Development Decisions. By J. D. Montgomery. Pp. 239. (MIT: London and Cambridge, 1974.) \$12.50.

and points out the relevance of certain developments in the contemporary context of developing countries.

A recurring theme in the book is the changing relationships among three modes of development politics—national planning, local options and individual choice—as forces that can direct the use of technology for advancing the quality of life in the undeveloped countries. This theme also provides the introduction to the theoretical structure of the work—a structure, it seems, that is deeply rooted in a behaviouralistic theory of politics. It is important to remember, cautions the author, that technological change implies changes in behaviour and that these cannot be brought about edict or *fiat* but must begin with a thorough grasp of the attitudinal *status quo* and build from that basis. But the modernisation of behaviour also cannot ignore the politics and structure of the community at the local level because individuals form an integral part of this structure and constitute its 'politics'. By the same

token, modernisation cannot be achieved in any significant way by the transformation of the community at the local level alone. Because technological development requires the re-orientation of all communities more or less simultaneously, unless it is directed at the national level modernisation will be a fragmented affair. Thus, the characterisation of the developmental problems is, in terms of these levels, intimately linked together. Accordingly, there are also three 'analytical orders' or levels of decision to be considered in applying technology to social purposes. The first order decisions are those which define and develop programmes for the purpose of benefiting identified elements of the society. The second are those that identify the administrative organisation or combinations of institutions that are to be assigned the role of moderniser and change agent. The third prescribes the incentive system by which administrators are to elicit modernising responses from citizens participating in the programme.

Because of its tight organisation and conceptual sophistication the book makes interesting and stimulating reading. Further, because even in advanced societies there are sectors which are becoming modernised or in need of becoming modernised, there is much in the way of insight and common sense for those in charge of regional development policies. □

SPEAKING as one with a personal interest in the engineering applications of holography, I found reading this book to be a refreshing experience. In addition to contributing personally, the editor has gathered contributions from 14 other internationally recognised experts in fields of optical measurement or display. Although holography features prominently the book is by no means confined to that topic nor is it just about non-destructive testing. The title is a bit artificial and seems almost to have been chosen as an excuse to bring this work together. In spite of that, the aspects of nondestructive testing do form a thread of continuity through a work on optical measuring techniques which are biased heavily to those which use lasers and to a lesser extent to those using coherent radiation in the form of microwaves or ultrasonics.

Any would-be user of techniques of

Optical measuring techniques

John N. Butters

Holographic Nondestructive Testing. Edited by R. K. Erf. Pp. xvii + 442. (Academic Press: New York and London, July 1974.) \$28.00; £14.00.

coherent radiation for measurement or testing would do well to read this book. It contains descriptions of practical test layouts and a wealth of experimental 'tips' which help to maximise the usefulness of experiments from a data retrieval point of view. Typical examples are given but some readers will be disappointed to find that these are based in the main on laboratory experiments. Contributions are sectionalised by the author and fall into two categories: substantive material which forms the core of the book; and reports

on significant developments. Sections in the first category contain mathematical backgrounds to the topics covered but there are some abrupt changes in style and technique. Presentation ranges from the quotation of relevant formula to fairly complex derivations extending to two or three pages. Readers wishing to gain benefit from the theoretical aspects would need a grounding in advanced mathematics.

All in all this is an excellent book for the serious user; it is well referenced and obviously emerges from a group well informed and well practised in their technology. Cited work is mainly of American origin so it may be appropriate to note that the techniques described are available in the UK at centres such as the National Physical Laboratory and the Mechanical Engineering laser facility at Loughborough University, to mention but two.

Nuclear techniques

Techniques in Nuclear Structure Physics. By J. B. A. England. Part 1, Pp. viii+1-312; Part 2, Pp. viii+313-697. (Macmillan: London and Basingstoke); (Halsted Press, distributors, USA, July 1974.) £10.00 boards; £4.50 paper.

IN recent years a wide variety of experimental techniques has been developed which allow measurements of increasing sophistication on the products of nuclear reactions. The techniques are well known to a few specialists but descriptions of them are often only available in scattered articles in specialised journals. The two volumes by Dr England thus fill a real need by providing a comprehensive summary of the more important techniques in a readily accessible form.

The first chapter is devoted to detectors and covers the photographic method, solid dielectric track detectors, gas ionisation chambers, scintillation and semiconductor detectors, spark chambers and Cerenkov and neutron detectors. There follows an account of instrumentation in general, including particle beam detection, measurement and handling, scattering chambers, electronics and on-line computers. The final chapter of Part 1 summarises various types of accelerators: the Cockcroft-Walton voltage doubler, the dynamitron, the Van der Graaf generator, insulating core transformers, linear accelerators, cyclotrons and cyclografs.

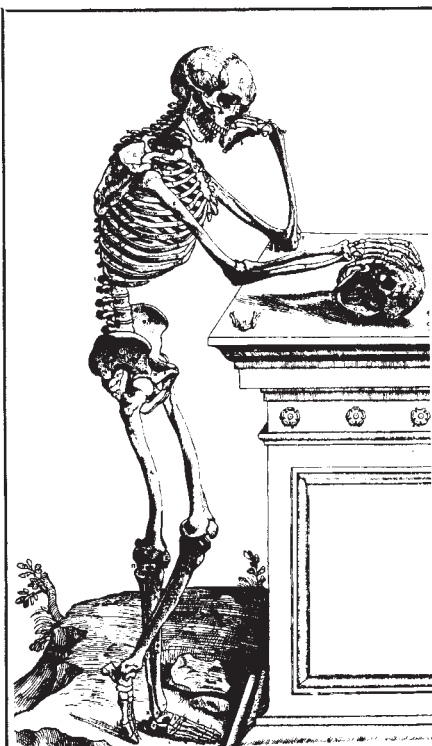
The second volume covers magnetic spectrometers and spectrographs, and particle identification techniques. Among the latter are time-of-flight techniques, techniques of single detector identification by telescope, and problems associated with passing detectors. Then comes a summary of coincidence measurements, angular correlations and lifetime measurements with accounts of the general theory of angular correlations, gamma-gamma and particle-gamma techniques, and correlations with oriented nuclei and nuclear lifetimes. The final chapter is devoted to polarised beams and polarised targets.

So numerous are the techniques today that even in a pair of volumes of some 700 pages it has been necessary to restrict the coverage by omitting the Mössbauer techniques, the techniques of beam-foil spectroscopy and the study of the capture γ rays produced by the interaction of polarised thermal neutrons. In other cases where good review articles are available, as in the subject of angular correlations, the discussion has been curtailed accordingly.

The level of the discussion is suitable for senior undergraduates and for first

and second year graduate students, and gives a clear account of the basic physics underlying each technique, together with many references to papers and monographs in which more detailed accounts can be found. There is a comprehensive list of references at the end of each chapter but, unfortunately, no alphabetical list of authors. The subject index is inadequate, so that it could take some time to find the account of a particular subject.

P. E. Hodgson



Taken from the frontispiece of the *Atlas of Human Anatomy*. Ninth English edition. By Frank H. J. Figge. (Original author: Johannes Sobotta.) Vol. 1: Regions, Bones, Ligaments, Joints and Muscles. Pp. xv+275. Vol. 2: Visceral Anatomy. Pp. xii+247. Vol. 3: Central Nervous System, Autonomic Nervous System, Sense Organs and Skin, Peripheral Nerves and Vessels. Pp. xii+354. (Urban and Schwarzenberg: Munich, Berlin, and Vienna, 1974.) No price.

Energy spectrum

Energy: Demand, Conservation and Institutional Problems. Edited by Michael S. Macrakis. Pp. xxvii+556. (MIT Press: Cambridge and London, 1974.) \$25.00.

To undertake the comprehensive reporting of 38 conference articles requires dedication, particularly when the modelling of energy systems is the subject of a major part of the work. The fact that Michael S. Macrakis achieved this objective in a hard backed volume of 530 pages within about a year is noteworthy.

The editor can be excused for rele-

gating another 28 articles, in abstract form, to an appendix for reasons of space, delayed submission, or because they were "addressed to solar energy". To add variety and interest the best of the articles on sunshine could have been included in full and the somewhat heavy going sections on aggregate and disaggregate modelling could have been reduced without great loss.

The sections of the book which include supply and demand, transportation, and conservation, provide helpful subdivisions for experts who wish to confine their reading. Even the energetics enthusiast would not, however, be impelled to read the book from cover to cover as it merits periodic random selection of a few passages for short perusal.

The reader interested in the prospects and problems facing a world trying to supply future energy needs can find half a dozen relevant papers. Another dozen contain useful data and can thus reduce searches elsewhere. A great deal of the data and subject matter is, however, of relevance purely to the United States.

Readers of this volume will quickly appreciate that national concern in the United States is having a positive effect.

Improved methods for energy production and application planning have been developed. There is a greater awareness of the factors affecting energy economics and the interrelation of the subject with environmental and real resource costs. There is also an appreciation of the need for a wider variety and depth in energy assessments, and there has been more concentrated research to improve energy technology.

It was good to note that a few European authors were able to contribute to the conference. A team from Queen Mary College, London, were very active in providing some interesting papers on world energy modelling, the implications of national policies on world energy, oil transportation studies, energy economics and atmospheric pollution. Several of these deal with components of the mathematical model being built up by the Energy Research Unit at Queen Mary College, which should become an increasingly useful tool for policy assistance in Britain.

But there are many practical technological problems to be solved and policy decisions to be made before the editor's introductory question is answered for each country operating within its own unique limitations. "How quickly", he asks, "with what set of energy sources, and at what prices can a supply of energy be secured, which satisfies environmental and safety constraints while accommodating prescribed economic paths?"

G. R. Bainbridge

Genetic Control of Insect Pests. By G. Davidson. Pp. ix+158. (Academic Press: London and New York, August 1974.) £4.00; \$10.25.

THIS book provides a useful overall statement of current and proposed methods for the autogenous control of agricultural and public health pests. It is well illustrated with 16 figures, 9 of which are photographs; and there are 359 references for further reading.

The opening chapters are concerned with the sterile male technique, still the only genetic control method with a claim to success (albeit temporary). The techniques for rearing, for example, 150 million flies a week make interesting reading. Radiation, chemosterilants and hybridisation as methods for producing sterile insects are covered in detail but there is no comparison of their relative merits. The more sophisticated genetic approaches proposed for controlling insects, such as chromosome manipulation, are thoroughly covered, and although much laboratory research is being devoted to them, field trials have not been too promising.

There is scant encouragement here for those who incline to the view that genetic methods will solve our pest problems and that the end of insecticides is nigh.

Although there is little discussion of future expectations for these approaches to pest and vector control, the book offers a succinct coverage of the subject to date with, perhaps, a bias towards medically important insects, in particular, mosquitoes; no doubt a reflection of the author's experience and interests. **I. Maudlin**

Biological Control by Natural Enemies. By Paul DeBach Pp. 323. (Cambridge University Press: London, 1974.) £5.50; \$14.95.

THIS is a timely and valuable book, written in characteristic style by one of the world's leading biological control workers. It highlights the disasters that have followed the heavy use of broad-spectrum pesticides and emphasises the highly advantageous cost-benefit ratio resulting from successful biological control projects. Numerous historical accounts of successful or partially successful campaigns are given, which highlight the colossal difficulties the early workers laboured under in the absence of global air transport, and the importance of intelligent sleuthing and intuition, as well as sound scientific understanding, in searching out the native ranges of natural enemies.

DeBach rightly emphasises the crucial importance of correct taxonomy and of seeking geographical races that are adapted to all the significant climatic and ecological situations occupied by

the pest. He points out that foreign exploration for natural enemies has not yet been attempted for more than 5% of the world's pests and it is certain that an even lower percentage of weeds has been considered for attack by this means. The potential of classical biological control therefore continues to be enormous.

DeBach makes a point that will bear emphasis: "How to put everything together to choose a potentially effective enemy *beforehand* with certainty is still beyond our grasp"; and further "... the natural enemy ... considered to be best often turned out to be inconsequential and, in fact, the best one even may be overlooked for years, as was *Cyrtorhinus*, the egg predator of the sugar-cane leafhopper." Although attempts to assess in advance the potential of biological control agents must

More campaigns in the pest war

continue (and they are gradually providing a basis for sounder choices), there is still no promise of a method for accurately forecasting the impact of a particular species. One could therefore join issue with Professor DeBach when he quotes liberally from Alvah Peterson in support of a plea for the study of the 99% of insects that are unimportant to mankind. There is not a shadow of doubt that biological control would languish indefinitely if significant proportions of its rather limited manpower and resources were diverted for this purpose. In this context it is fortunate that there is little evidence that the presence of relatively ineffective introduced enemies hampers the activities of effective agents.

In addition to the great economic potential remaining in 'classical' biological control there are also prospects for the artificial augmentation of populations of natural enemies in relation to certain problems, as when parasites and predators are bred commercially in large numbers for pest control in orchards, poultry houses, dairies and feedlots.

The author also deals briefly with other non-chemical forms of pest control, such as the use of resistant plants, cultural and genetic methods, and pheromones, and he gives some useful discussion on integrated control and on the intelligent and selective use of pesticides where that is unavoidable.

This book is obviously not intended as a comprehensive treatise on biological control—indeed the vast majority

of references are to American publications—but it gives a generally useful philosophy on applied entomology which all interested research workers should study. **D. F. Waterhouse**

The Biochemical Mode of Action of Pesticides. By J. R. Corbett. Pp. ix+330. (Academic: London and New York, 1974.) £7.20; \$18.50.

ANY author attempting to describe what is known about the mode of action of poisons faces an unenviable yet stimulating task, for information on the molecular basis of normal physiological activities is frequently incomplete. Impulses pass along nerves by the opening of ionic 'gates' but little is known of the mechanism operating through those gates. The same applies to the coupling of electron transport with phosphorylation in mitochondria and chloroplasts, to the biochemical basis of auxin action, and to the mechanism of oxygen release in the second light reaction of photosynthesis.

As a majority of pesticides probably affect such processes, any account of their action must, of necessity, be incomplete. Dr Corbett is to be congratulated on one of the best attempts so far made to collate available evidence and to present it in a way comprehensible to all biologists possessing a background knowledge of biochemistry and supporting chemistry. It is a valuable reference book for the undergraduate library collection but is not likely to be recommended for private purchase by students of agriculture or horticulture.

The book comprises three main sections covering compounds acting respectively, on photosynthesis, nerve action and growth. They include tables of pesticides, which are valuable sources of information. The tables would have been even more useful, however, if some indication could have been given of the relative practical importance of the hundreds of compounds they contain.

An author tends to provide space according to the information available on a group of compounds, rather than in proportion to economical importance. Dr Corbett cannot, therefore, be criticised seriously for the fact that some 30 pages are devoted to the useful but locally important urea and triazine weedkillers whereas the crucially important phenoxyalkanoic acids are dismissed in five text pages. Similarly, although it is grand to encounter an author with the courage to face the unpopular truth that DDT (despite its manifold faults) has probably saved more lives than any other chemical made by man, it is a pity that it is dismissed in about eight pages.

K. A. Hassall

Bird communities

Competition and the Structure of Bird Communities. By M. L. Cody. Pp. viii+318. (Monographs in Population Biology, No. 7.) (Princeton University: New Jersey, July 1974.) \$6.60 cloth; \$3.65 paper.

Cody's book is of more general interest than the title would indicate for he has attempted, with some success, to effect a synthesis between the data of field biologists and the niche and competition models of theoretical ecologists. It will be of interest even to ecologists who hate birds. Cody emphasises the importance of "natural experiments" in understanding community structure, and he makes informed comparisons of mainland and island bird communities, and of California chaparral and Chile matorral bird communities.

He presents a great deal of data that are consistent with the predictions of the theory of community structure developed by the late Robert MacArthur. For example, Cody finds that variation in foliage height diversity accounts for 81% of the variation in bird species diversity in eight North American bird communities (page 128). Cody's extensive field data point out some of the weaknesses of current theory: suppose that one can measure accurately the amount of competition for food, and that one can measure accurately the amount of competition for habitat. How can they be combined into one number that reflects the total amount of competition between two species? If competition along different niche axes (food, habitat, and so on) is independent, then the total amount of competition is the product of the competition coefficients along the different axes. This assumption of independence has usually been made by theoretical ecologists, but Cody found that addition, rather than multiplication of the competition coefficients along the different axes made for better predictions of community structure.

At times I found the book frustrating because Cody did not develop some of his insights more thoroughly. For example, Cody repeats and partially justifies one sacred cow of ecology—'jack-of-all trades and master of none' (page 55), but on page 68 he shows that species that occupy a broad habitat range often eat a broad range of foods, and argues that this is optimal. Such a species would seem to be a 'jack-of-all trades and master of some'. Another example is his demonstration of character convergence, which he argues is a consequence of interspecific competition. Yet he does not discuss or test the model of Mac-

Arthur and Levins that predicts character convergence when a species invades a community in which resource overlap between species is high. Instead, Cody discusses the relationship with interspecific territoriality, but this seems to confuse cause and effect.

Readers not convinced that competition is a major factor in community organisation may not be persuaded by Cody's book. In some cases, alternative explanations are available for the phenomena Cody attributes to competition: for example, geographical separation of closely related species. Nevertheless, Cody presents a large diverse body of data consistent with competition theory. Any refutation of the importance of competition in determining community structure must account for the extensive observations Cody has made. **David C. Culver**

Carbon and light

Organic Photochemistry. By J. M. Coxon and B. Halton. (Cambridge Chemistry Texts). Pp. vii+196. (Cambridge University: London, 1974.) £4.20; \$13.00.

ALTHOUGH several new books on organic photochemistry, aimed mainly at senior undergraduate and graduate students, have been published in the last two or three years the subject is undergoing such rapid expansion that new titles are always welcome. The book under review is divided into five chapters dealing with an introduction to photochemistry; intramolecular reactions of olefinic bonds; intramolecular reactions of carbonyl compounds; cycloaddition reactions; and substitution, oxidation, and reduction. The text is commendably free from typographical errors.

One of the many problems confronting authors in the area of organic photochemistry is the quantity of scientific literature to be covered. There is no possibility of encyclopaedic coverage in a small volume but the information cited should at least be correct. Unfortunately, this is not always so in this book. On page 140 it is stated that 2-methoxynaphthalene affords a (2+2) dimer involving the 9,10 bonds, though, in fact, the dimer formed is of the (4+4) type involving addition across the 1,4 sites of the substituted ring. This structure has been verified by X-ray analysis (*Chem. Commun.*, 978; 1969).

On the whole the authors have kept this book up to date although, inevitably, since the publication date new results have out-dated some of the reaction mechanisms. The book is very readable and gives coverage suitable for the students at whom it is aimed. **William Horspool**

Ising model

The Two-Dimensional Ising Model. By Barry M. McCoy and Tai Tsun Wu. Pp. xvi+418. (Harvard University: Cambridge, Massachusetts; Oxford University: London, February 1974.) £12.50.

THE two-dimensional Ising model gives a simplified description of a wide range of physical systems including fluids, magnets and binary alloys; without question it is the most thoroughly investigated model in statistical mechanics. Its main interest is that, though simply formulated, it has a surprising richness and complexity, and throws much light on the behaviour of systems undergoing a phase transition. In 1944 L. Onsager performed the major feat of calculating the partition function of the square Ising lattice in zero field, and found some of its thermodynamic properties. The results profoundly changed the theory of phase transitions and critical behaviour.

Onsager's calculation and later investigations by others were notorious for their difficulty, and this made the study of the Ising model practically a branch of theoretical physics by itself. In 1962 the Dutch physicist P. W. Kasteleyn achieved considerable simplification by showing that the two-dimensional model in zero field is equivalent to the combinatorial problem of placing dimers on the bonds of the lattice such that each lattice point is covered by the end of one dimer, and counting the number of ways in which this is possible. This problem has a simple solution in terms of the Pfaffian of a matrix which can be evaluated without much difficulty. Over the last 12 years the method has become a powerful tool by which many more detailed properties of the model have been evaluated. The authors of the present monograph have made notable contributions in this field. Even though the method is simple in principle, research papers still tend to be hard reading because of their conciseness and the amount of detail. The aim of this book is to give an elaborate exposition of the dimer method and its application in various situations. It does not present a comprehensive survey of all the work which has been done on the two-dimensional Ising model. The authors have succeeded admirably in their limited aim and have given a careful exposition of the mathematical techniques involved and the results obtained. For those who want to be acquainted with the model and its history some existing reviews would seem preferable. But this book is recommended to anyone aspiring to research in the area and wishing to acquire a working knowledge of the dimer method. **B. U. Felderhof**

This work is a detailed source book of the structure of all phosphorus compounds that have been reported up to 1972, with a footnote to each chapter, added in proof, to extend the scope to early 1973. The information presented covers that derived mainly by X-ray diffraction, nuclear magnetic resonance and infrared spectroscopic techniques, with the emphasis strongly on the first of these three. The author restricts himself to a comparative discussion and establishes the range of molecular dimensions to be found within various classes of compounds. Though the results are briefly interpreted, the strength of the book is in the results themselves.

The first chapter outlines the general bonding properties of phosphorus and contains an essential and salutary analysis of the reliance that can be placed on the molecular dimensions derived from X-ray structure determinations. The second chapter is devoted to the many structures of phosphorus and this complex story, very neatly exposed, leads naturally to the next chapter on phosphides, where the structural diagrams will, perhaps inevitably, only be easily understood by crystallographers.

Chapters 4-9 (202 pages) cover the compounds in which phosphorous is mainly bonded to elements in group six, among which oxygen predominates. A short survey of oxides, sulphides and selenides is followed by a longer discussion on orthophosphates. In these extended structures, as with the phosphides, the structural diagrams are not always easy to interpret. The condensed phosphates are ex-

amined in chapter 6 where this complex series of compounds are elegantly classified by their structures.

The phosphate esters are covered in chapter 7 which contains a lengthy digression on DNA and is one of the less satisfactory sections of the book, a reflection perhaps of the meagre structural information available for this class of compounds.

Chapter 8, on substituted phosphates, is lucid and concise and the structures of the isolated molecules that make up the greater part of this category lend themselves to the clear repre-

phorus are found in two chapters—12 and 14—with the first restricted to a simple survey of the structural chemistry of the phosphazines and the second swiftly covering all the remaining ring compounds. Curiously inserted between these two chapters is one on isomerism and optical activity.

The final chapter on cage structures is very short: few containing phosphorus have been reported, and the text is padded somewhat with analogies to cage systems formed by atoms other than phosphorus. There are appendices with a list of unit cell and spacegroup data for phosphorus compounds, an infrared correlation chart and NMR chemical shifts for typical compounds, and 2,649 references.

The book is printed on good quality paper with an elegant typographical balance between text and diagrams. It must have been a proof reading nightmare, but though few errors remain, it is disconcerting to find that a diagram of vitamin B₁₂ (labelled vitamin A) has a structural formula with two very uncanonical carbon atoms.

Throughout the work the author not only exercises a balanced judgement on his source material but presents the evidence on which he bases his judgement. The book will thus be a valuable aid and happy hunting ground for those working in the field of structural chemistry of phosphorus while still being able to supply single items of information, in context, with an evaluation for those with less specialised requirements. Despite its high price there will be few research laboratories that would want to be without access to this book.

Phosphorus chemistry

T. S. Cameron

The Structural Chemistry of Phosphorus. By D. E. C. Corbridge. Pp. xiii + 542. (Elsevier: Amsterdam, London and New York, 1974) Dfl.250; \$96.20.

sensation they receive. The behaviour of bonds between phosphorus and oxygen atoms is examined in chapter 9 where a discussion on hydrogen bonding is concluded by a summary of P-O bond length (and interbond angles) over the range of compounds described in the previous five chapters.

Hydrides, nitrides, halides and phosphines are the subject of chapter 10 and the following chapter covers the compounds where phosphines act as ligands with metal atoms. There, with a vast range of material the author has little alternative but to compile a catalogue of the known structures. Details of the ring compounds of phos-

Crystal chemistry

The Major Ternary Structural Families. (Crystal Chemistry of Non-Metallic Materials.) By O. Muller and R. Roy. Pp. ix + 487. (Springer-Verlag: Berlin and New York, 1974.) DM76; \$31.10.

In his introduction to this first volume in the series of publications on the crystal chemistry of non-metallic materials, Professor Rustum Roy comments on the fact that few books have been published on the subject of crystal chemistry. If the forthcoming volumes in the series maintain the high standard set by this issue much of the deficit will have been made up.

The introduction is extremely comprehensive and makes certain that the reader understands how to make fullest use of the large amount of information given in the subsequent chapters. Frequently, introductions seem to have been written as an afterthought but

here one senses that considerable care has been taken with what should be a most important chapter.

Three major structural families are dealt with in detail: A₂BX₄, ABX₄ and ABX₃; with a small number of other structures collected together in table form at the end of the book. Each major structural family is subdivided into individual structural types based on a particular compound. Summaries at the start of each chapter indicate clearly the range of structures covered, thus enabling the reader to locate rapidly any structure of particular interest. Of especial value is the grouping of all the individual structure types into a structural field map at the end of every chapter. The reader can then appreciate to the full the interrelationships of each structure with those of others in the same group. All of the diagrams in the book are of very high quality.

A problem for any author writing on crystal chemistry is how best to pre-

sent the large amount of crystal data which are required. In this book the authors have chosen to place nearly all such data, in tabular form, into an appendix. Some selected data necessary for an understanding of the text is tabulated in each chapter. The result of this is, however, that almost half of the book is made up of the appendix.

There are two small, but pleasing, aspects of this book: first, the comprehensive and easily found tables of ionic radii, which may well be consulted more frequently than many of the tables; and second, the short sections on the applications of some of the compounds mentioned.

The volume is an excellent reference work and perhaps my only criticism is that the range of structures covered is limited; but one can appreciate that any volume which attempted to cover, in this amount of detail, the whole range of ternary structures would become overlong and unreadable.

M. G. Barker

Radically speaking

Microwave Spectroscopy of Free Radicals. By Alan Carrington. Pp. ix+264. (Academic: London and New York, June 1974.) £4.80; \$12.50.

THIS is a highly specialised monograph by one of the major workers in the field discussed. As expected, it is clearly and precisely written and will be invaluable to those interested in, or working in, that field. It is not, however, likely to be of great interest to conventional electron spin resonance (e.s.r.) spectroscopists, nor is it, in any sense, a text on microwave spectroscopy as such.

The term 'microwave spectroscopy' is extended to cover all experiments "in which the absorption or emission of microwave radiation by a molecular species can be detected directly or indirectly". The term 'free radical' is extended well beyond the normal doublet state species to include $^1\Sigma$, $^1\Pi$, $^1\Delta$, $^3\Sigma$ and $^3\Pi$ states. Thus, the experimental section includes sections on microwave rotational spectroscopy, gas-phase electron resonance, microwave/optical double resonance, and molecular beam spectroscopy.

Similarly, the 'free radical' species include a wide range of diatomic species such as CS, AlF, H₂, O₂, SO, SeO, NF, CN, and the small number of triatomic molecules so far studied, namely NCO, NCS, HNO, CF₂, SiF₂, HCO, DCO, NO₂, ClO₂.

The author starts by asking if perhaps this specialised field should be viewed as a "backwater": that it should not be is well demonstrated in this excellent book.

Reactive Free Radicals. By J. M. Hay. Pp. viii+158. (Academic: London and New York, June 1974.) £4.40; \$11.50.

THIS book should have a far more explicit title such as 'Structure-reactivity relationships for Organic Radicals'. The author is very much concerned with kinetic parameters derived from reactions in the gas phase. I suspect that the treatment is too biased towards the author's particular conceptions to make it useful for undergraduates, but other workers should find it stimulating.

In the preface we are warned that the book is based on views that are "perhaps even naive" and I fear that this may be a valid warning. Nevertheless, I enjoyed reading it and found several suggestions well worth toying with. I must say, however, that I found an awful lot of glib statements to which I would take exception, such as, "charged radicals are normally produced by electron-transfer at electrodes or from charged species". Or we are told that high-energy radiation pro-

duces an "embarrassment of products" (if that were always true, I would be out of business). Then we are told that electron spin resonance (e.s.r.) spectroscopy will not detect pairs of radicals formed in solvent cages, despite the fact that one of the most important e.s.r. developments in recent years has been the study of such 'triplet' species.

One very misleading suggestion is that the breaking of the C-C bond in R₃C-CR₃ molecules may result in the formation of σ -radicals R₃C-sp³-hybridisation is implied—despite the fact that the author accepts that R₃C-radicals are planar or very nearly so. Even more unfortunate is the fact that e.s.r. evidence in favour of this idea is cited, although this has long since been refuted. Again, we are told that a proton hyperfine coupling of ± 16 gauss for HC≡C· is "hard to reconcile with a linear structure". Why?

Throughout, very simple valence-bond structures are presented (often consuming a lot of space) and the results of sophisticated molecular orbital calculations are completely ignored. I can see very little justification for such an approach in a study which is primarily concerned with structural problems.

This short book in no sense overlaps with the excellent recent work on radical displacements by Ingold and Roberts, despite the title. The presentation is satisfactory except that references are given at the end of each of the five chapters. As no help is given to the reader, one inevitably turns up the wrong set of references which can prove to be very misleading. Please can we have all references at the end?

M. C. R. Symons

Nervous systematics

Evolution of the Nervous System. By Harvey B. Sarnat, and Martin G. Net-sky. Pp. xvii+125. (Oxford University: London and New York, September 1974.) £5.75 boards; £4.00 paper.

THIS is one of the very few comparative anatomy textbooks of reasonable textbook size. It explores neuro-anatomy, system by system, in a functional way "through the dimension of time as revealed by analysis of evolution". The book starts with a general overview of vertebrate evolution, and then proceeds with a system by system analysis, including the brain, spinal cord, and the autonomic nervous system. The emphasis is always on how the evolutionary series relates to the structural and functional organisation of the human brain. The book does not cover invertebrate nervous systems, nor those aspects of vertebrate evolution which are divergent from the point of view of the human nervous system. Written by

a neurologist and a neuropathologist, the text brings out wherever possible the bearing of the comparative approach on our understanding of various congenital and pathological conditions in the human nervous system as encountered in clinical practice. The authors do not hesitate to deal with provocative and speculative points, although always making it clear where they are being speculative.

There is an extensive biography, and the references are reasonably up to date in the majority of the sections. The text, however, lacks references to recent developments in the study of central biogenic amines, especially in the accounts of the locus coeruleus, hypothalamus, and nigrostriate system. There is an interesting atlas of selected brain sections in different species.

This comparative neuroanatomy textbook is neatly and handsomely bound, and moderately priced. The text is highly readable and, as all the basic anatomical arrangements are described, it is complete in itself and does not require additional reference to a standard neuroanatomy text. The chapters are well indexed, and within each chapter the sections are clearly headed and subdivided. This makes it easy to look up any particular system, and enhances the value of the book as a reference work.

G. Raisman

Tropical forests

Tropical Forest and its Environment. (Tropical Ecology Series.) By K. A. Longman and J. Jenik. Pp. x+196. (Longman: London, June 1974.) £1.95.

THIS lucidly written and useful booklet belies its ambitious title, for, as stated in its preface its principle contribution is a synthesis of results of physiological experiments on Ghanaian forest trees carried out in controlled environments, and parallel field investigations; both are then related to existing knowledge of rain forest ecology. The careful accounts of the authors' experiments provide new and rewarding insight into West African rain forests, but attempts to relate them to forests elsewhere in the tropics, and more particularly the additional general chapters on subjects unrelated to the experiments, are of variable quality, as the authors too often assume, wrongly, that West African forest conditions are universal in the humid tropics. The account of tropical forest soil, for instance, is misleadingly over generalised; that on biotic factors is so superficial that it would have been better omitted; previous data is sometimes misquoted (notably in Fig. 4.9); and much recent literature is left unconsulted. But there are well designed diagrams and 25 pages of photographs.

P. S. Ashton

obituary

M. Ewing

MAURICE EWING, the eminent oceanographer, died in Galveston, Texas, on May 4, 1974.

Ewing attended the Rice Institute (now Rice University) for both his undergraduate and graduate education in Physics. On receiving a Ph.D. in 1931, he was appointed to the Physics Department at the University of Pittsburgh, then at Lehigh University where William Bowie, Richard Field and Walter Bucher introduced him to the problems of seagoing geophysics. Using equipment and help from F. A. Vening Meinesz, pendulum gravity measurements at sea were begun. With his students (and lifelong associates) Vine, Webster, Woollard and Worzel, techniques were developed for making seismic measurements with the explosive source and automatic recording equipment on the seafloor. In this manner, the first marine seismic refraction measurements were attained just before the outbreak of the Second World War. Sediments were found to be thicker over the continental shelf than in the deep sea.

In 1940 Ewing took leave of absence (he was then an Associate Professor of Geology at Lehigh) to move with his group to the Woods Hole Oceanographic Institution and work on problems of submarine detection in association with Columbus Iselin, who had become impressed with the decisive role played by the thermocline in determining sound transmission characteristics in the upper ocean. Ewing and Vine adapted the Spillhaus bathythermograph for use on vessels while underway. Iselin, Ewing and Worzel produced a manual, *Sound Transmission in Sea Water* for use in a basic training course at Woods Hole for Naval Officers. Ewing and Pekeris were the first to recognise the importance of the minimum in sound velocity for long-range sound transmission (SOFAR). The expected wave guide effect was demonstrated with a dramatic test: a small explosive was detonated at depth in the eastern Atlantic, and recorded on the western side with hydrophones suspended into the SOFAR channel. Ocean bottom photography, developed as a sideline in previous years, was applied to underwater shipwreck photography. With some incredible luck, Ewing obtained a picture of a wreck's nameplate; he would carry this in his

pocket to convince sceptical Naval officers of the usefulness of underwater photography for identification purposes. The first seabottom wave recorder was built by Ewing.

In 1946 Ewing and some of his group returned to academic work—this time at Columbia University, where they soon founded the Lamont Geological Observatory (now Lamont-Doherty). Ewing served as Associate Professor of Geology, then Professor, occupying the Higgins Chair from 1959 to 1972. Under Ewing's direction, the Lamont Observatory moved into the forefront of geophysics and geology, especially as related to the oceans. Seismic refraction measurements were resumed, this time not with bottom-dropped instruments but with suspended hydrophones. Ewing developed and used towed magnetometers (first flux-gate, later nuclear precession), and initiated continuous reflection profiling with air-gun sources. He improved techniques for precision depth recording, gravity measurements (from submarines and later from surface vessels) and long piston coring, and carried out heat-flow measurements in conjunction with piston coring.

Yet another contribution was the suggestion that turbidity currents played a vital role in sedimentary processes. The study of the sedimentary evidence led to a theory (with Donn) for climatic change.

In association with Frank Press and Wenceslas Jardetsky, Ewing launched a major era of seismology with the study of surface wave dispersion, explaining many previously misunderstood features of seismograms. The book *Elastic Waves in Layered Media* by Ewing, Jardetsky and Press served as a basic text for a new generation of seismologists.

Thus, Ewing's career was one of many thrusts into the unknowns of the Earth: the Continental Shelf, submarine ridges and trenches, the structure of continents, ocean basins and the earth as a whole, the structure and origin of sediments and the structure of the oceanic water column. He certainly provided plenty of stimulating competition for other oceanographic laboratories.

The development of the theory of ocean floor spreading and plate tectonics was made possible to a great extent by the observations made by Ewing and his coworkers. The dis-

covery of the thinness of the oceanic crust and oceanic sediments and the discovery of the continuity of the mid-oceanic ridge system were especially important keys. Ewing enthusiastically supported the JOIDES drilling programme which was to give dramatic confirmation to the concept of ocean floor spreading. He maintained a healthy scepticism for any new schemes proposed either by himself or others and the theory of the new global tectonics was no exception. He was later to comment that he was amazed to see so many of the facts that had puzzled him about the oceans explained by such a simple theory.

Ewing was a man of quiet courtesy, which belied a singlemindedness of purpose. Once the priorities were set, nothing would be permitted to get in the way. Thus, he would turn the lathe in the ship's cramped machine shop all night to replace a faulty gear. Once when swinging gravity pendulums aboard submarines, he told one of us: "If we get sub time, no matter where or when, Worzel or I will be aboard." While securing some explosives aboard the VEMA during an early morning gale, Ewing and two shipmates were washed overboard. Ewing and one man were miraculously saved but Ewing sustained injuries from which he never quite recovered. In reflecting upon his own reactions, Ewing characteristically assigned his priority: to stay afloat.

During his long and productive career, he helped train more than 200 graduate students. Affectionately known to his students as 'Doc', he expected no less from them than the hard work and devotion that he himself was always ready to give. His students and colleagues will remember the many times they stepped from his office inspired and challenged by either a few minutes or a few hours of intense interaction. He was the author of more than 300 papers and many honours came to him: among them, election to the National Academy of Sciences in 1948, the Vetlesen Prize in 1960, Foreign Membership of the Royal Society in 1972, and also the National Medal of Science in 1973.

His last two years were spent very happily as Green Professor of Marine Sciences and Founding Chief of the Earth and Planetary Sciences Division of the University of Texas Marine Biomedical Institute in Galveston.

Announcements

Awards

Philip S. Corbet has been awarded the Gold Medal Award of the **Entomological Society of Canada**.

The **Fondation de Physiopathologie Professeur Lucien Dautrebande Prize** has been awarded to **Professor Durrer** for his contribution to clinical and electrophysiological knowledge of the heart.

Judah H. Quastel, Hans J. Müller-Eberhard, Hector F. DeLuca, Roger Guillemain, Andrew V. Schally, David Baltimore and **Howard M. Temin** are recipients of the **17th Annual Gairdner Foundation Awards**.

Robert Allan Smith has been made an honorary Commander of the Order of St Olav, the Royal Norwegian order of chivalry.

Appointments

James Barron Bridges has been appointed to a second chair of physiology at the **Queen's University of Belfast**.

Philip John Randle has been appointed to the chair of clinical biochemistry at the **University of Oxford**.

Malcolm Rowland has been appointed to the additional chair of pharmacy at the **University of Manchester**.

Miscellaneous

The **Pharmaceutical Manufacturers' Association Foundation Medical Student Fellowships**. Four one-year grants for the academic year beginning July 1, 1975, will be awarded to candidates interested in research and teaching careers in pharmacology/clinical pharmacology.

The Foundation will also award two-year postdoctoral fellowships in pharmacology/morphology. For information contact: **Thomas E. Hanrahan, Pharmaceutical Manufacturers' Association Foundation**, 1155 Fifteenth Street, N.W., Washington DC 20005.

Maltwood Fund for Archaeological Research in Somerset. The **Royal Society of Arts** invites applications for grants in aid of archaeological research to be carried out in the county of Somerset during 1975. For further information contact: **J. S. Skidmore, Royal Society of Arts, John Adam Street, Adelphi, London WC2 6EZ**.

International meetings

December 9, Imaging by Holography, London (R. J. Neville, Research Division, Kodak Ltd, Headstone Lane, Wealdstone, Middlesex).

December 11–13, Surface Analysis, Slough (D. Nicholas, Head of Analytical Services, Fulmer Research Institute Ltd, Stoke Poges, Slough SL2 4QD).

December 22, Ethics in an Age of Pervasive Technology, Israel (Dr Mordechai Levy, Senate Building, Technion Haifa, Israel).

January 3, Elucidation of Important Mechanisms for Controlling Cellular Integrity, London (Dr D. J. G. Davies, School of Pharmacy and Pharmacology, Claverton Down, Bath BA2 7AY).

January 6–8, The Mechanics and Physics of Fracture, Cambridge (Meetings Office, The Institute of Physics, 47 Belgrave Square, London SW1X 8QX).

January 6–9, Geophysics and Cosmology of Solar System Observables, Newcastle-upon-Tyne (Dr F. R. Stephenson, School of Physics, The University, Newcastle-upon-Tyne NE1 7RU).

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